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Genetic variation in the folate metabolic pathway and risk of childhood leukemia

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Abstract

Studies of childhood leukemia and the potential etiological role of genetic variation in folate metabolism have produced conflicting findings and have often been based on small numbers. We investigated the association between polymorphisms in key folate metabolism enzymes (*MTHFR* 677 C>T, *MTHFR* 1298 A>C, *SHMT1* 1420 C>T, *MTR* 2756 A>G, *TS* 1494del6 and *TS* 28bp repeat) in 939 cases of childhood acute lymphoblastic leukemia (ALL) and 89 cases of acute myeloid leukemia (AML) recruited into the United Kingdom Childhood Cancer Study. We also examined the maternal genotypes of 752 of these cases. Data from 824 non-cancer controls recruited were used for comparison. No evidence of an association with *MTHFR* 677 was observed for ALL or AML, either in children or their mothers. However, in children an increased risk of ALL (OR 1.88, 95 % CI 1.16-3.07 p=0.010) and AML (OR 2.74, 95% CI 1.07-7.01 p = 0.036) was observed with the *MTR* 2756 GG genotype; the association most pronounced for cases with the *MLL* translocation (OR 4.90, 95% CI 1.30-18.45 p = 0.019). These data suggest that genetic variation in methionine synthase could mediate risk of childhood leukaemia, either via effects on DNA methylation or via effects on fetal growth and development.

Introduction

Leukaemia accounts for around a third of all malignancies diagnosed in childhood with approximately 57000 cases reported worldwide each year. The major morphological subtypes of leukaemia, acute lymphoblastic (ALL) with a B-cell precursor phenotype and acute myeloid leukaemia (AML), are characterised by gross chromosomal abnormalities [1 2], several of which have been shown to originate in utero [3-6]. Although there has been much speculation about the nature of the potential agents that could cause such alterations there is, as yet, no consistent evidence to support a link with either specific exposures or modifiers of exposure[7]. Folate levels along with genetic regulation of folate metabolism have been the focus of many investigations [8-19], predicated on the notion that they may influence the creation and/or expansion of the pre-leukaemic clone via DNA hypomethylation of key regulatory genes as well as uracil misincorporation into DNA leading to double strand breaks and chromosomal aberrations [20;21].

A critical component of the folate metabolic pathway is methylene tetrahydrofolate reductase (MTHFR) which controls the balance between DNA methylation and synthesis via the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-MeTHF), required for DNA synthesis, to 5-methyl tetrahydrofolate (5-MeTHF), a methyl donor for conversion of homocysteine to S-adenosyl methionine (SAM) (Fig. 1). Two common polymorphisms in *MTHFR* (677 C>T and 1298 A>C) which result in decreased catalytic activity [22;23] and subsequent availability of 5,10-MeTHF and SAM, have been extensively studied in relation to childhood leukemia, but findings have been inconsistent. However, *MTHFR* is only one of over 30 different enzymes involved in this pathway, and functional polymorphisms in other key enzymes such as methionine synthase (*MTR*), thymidylate synthase (*TS*) and serine hydroxymethyltransferase 1 (*SHMT1*) have been shown to moderate the risk of haematological malignancies [24-26]. These polymorphisms include *MTR* 2756 (A>G) which moderates the flux of single carbon moieties for DNA methylation processes [27]; *SHMT1* 1420 (C>T) which reduces circulating folate levels thus shunting 5,10-

MeTHF towards DNA synthesis [28]; a 6bp deletion (1494del6) in the 3' UTR of *TS* that influences RNA levels [29]; and a polymorphic tandem 28bp repeat sequence within the promoter enhancer region of *TS* where the triple repeat increases gene expression levels and reduces DNA damage [30].

With a view to providing further insight into the association between childhood leukaemia and folate metabolism we analysed polymorphisms in *MTHFR, MTR*, *SHMT1* and *TS* in over 1000 cases of acute leukaemia and their mothers recruited as part of the United Kingdom Childhood Cancer study (UKCCS).

Methods

Study population

Cases were children aged 0-14 years diagnosed with leukemia between 1991 - 1996 recruited into the UKCCS [31]. Samples taken at the time of diagnosis underwent immunophenotype and cytogenetic analysis [31;32]. Specific chromosomal abnormalities including *MLL* lesions, *TEL-AML1* translocations and hyperdiploidy were identified by a combination of banded karyotyping, RT-PCR and fluorescence in situ hybridization where appropriate [32]. In addition, peripheral blood samples were taken in remission from which DNA was extracted for this and other genetic studies [31;32]. In total, DNA was available for 1028 Caucasian cases (55.2% male) of which 939 (91.3%) were ALLs and 89 (8.7%) were AMLs. Of the 939 ALLs, 765 (81.5%) were B-lineage (738 pre-cursor B-cell and 27 pro-B cell) and 87 (9.3%) were T-lineage in origin. The remaining 87 (9.3%) were not entered into clinical treatment trials and details of their immunophenotype were not recorded. With respect to common cytogenetic groups, genotype data were available for 103 cases with a *TEL-AML1* translocation (52.4% male), 316 cases with hyperdiploidy (56.7% male) and 34 cases with an *MLL* lesion (35.3% male). Maternal DNA was available for 752 of the leukemia cases, which included 685 ALLs (573 B-lineage and 54 T-lineage) and 58 AMLs. DNA was amplified using DNA polymerase Phi-29, which has been previously validated for use in genetic epidemiology studies [33;34]. As part of routine quality control procedures, we also compared pre- and post amplified DNA genotyping results on a random sample set.

DNA was obtained from peripheral blood samples taken from 824 non-cancer Caucasian controls (54.1% male) selected from population registers as part of a UK-based case-control study. DNA from this control series has been included in several genetic association studies [25;35]. Both the UKCCS and the case-control study from which the controls were obtained were carried out with approval from the UK multi-regional ethics committee and in compliance with the Declaration of Helsinki.

Genotyping

Genotyping was carried out using TaqMan Assays-by-Design™ supplied by Applied Biosystems (ABI) (Applied Biosystems, Foster City, CA) with probes and primer sets for *MTHFR* 677C>T (rs1801133), *MTHFR* 1298A>C (rs1801131), *SHMT1* 1420C>T (rs1979277), *MTR* 2756A>G (rs1805087), and *TS* 1494del6 (rs16430) polymorphisms and the protocol for the *TS* 28-bp repeat identical to those previously published [26;36]. Case samples were genotyped for all six polymorphisms, whereas analysis of mothers samples was restricted to *MTHFR* 677C>T, *MTHFR* 1298A>C and *MTR* 2756A>G. TaqMan genotyping assays for *MTHFR* were verified by running 96 Coriell samples of known genotypes (http://snp500cancer.nci.nih.gov). All other TaqMan assays were verified by direct sequencing or using standard restriction fragment length polymorphism analysis. For added quality assurance, 5% of control samples were selected at random for repeat analysis, four independent control samples were analyzed on each 96-well plate, thirty duplicate DNAs were randomly distributed across the entire plate series and three duplicate plates were included in each genotype analysis.

Statistical Analysis

Estimates of the odds ratios (OR) for having leukemia were obtained for each polymorphism using univariate logistic regression models (Genmod procedure)[37]. Genotypes were considered as classes in the regression models so there was no pre-determined expectation of a particular dose-response relationship between the number of variant alleles and the risk of having leukemia. Bivariate gene-gene interactions were assessed by adding multiplicative interaction terms between pairs of genes, one at a time, to a multiple logistic regression model that included all of the genes as covariates. Only individuals with non-missing genotype data for all six polymorphic sites were included in the multiple regression. To be included in a specific analysis, individuals must have had non-missing genotype information for all SNPs included as covariates in the regression model being assessed. Associations in the distributions of

gene polymorphisms in pairs of genes were assessed separately for cases and controls using a series of chi-squared tests.

Results

Genotype distributions for leukemia cases and controls and are shown in Table 1. The control frequencies for *MTHFR* 677C>T, *MTHFR* 1298A>C, *SHMT1* 1420C>T, *MTR* 2756A>G, *TS* 1494del6 or *TS* 28-bp repeat were all in Hardy-Weinberg equilibrium (data not shown) and are similar to those reported in other Caucasian populations [10;11;14-18 24;36].

No statistically significant case-control differences in the distribution of the *MTHFR* 677C>T, *SHMT1* 1420C>T, or *TS* 28-bp repeat polymorphisms were observed for ALL or AML (Table 1). However, a dose-response relationship between the numbers of copies of the *MTR* 2756 G-allele and increased risk of ALL, specifically that of B-lineage ALL, as well as AML was observed (Table 1). Specifically, heterozygosity (AG) was associated with a 1.24 fold increased risk of ALL (95% CI 1.00-1.53 p=0.05), and homozygosity for the variant allele (GG) with a 1.88 fold increased risk of ALL (95% CI 1.16-3.07 p=0.01) and 2.74 fold increased risk of AML (95% CI 1.07-7.01 p=0.036). Findings were similar for B and T-lineage ALL. In addition, homozygosity for the *TS* 1494del6 polymorphism (6bp-/6bp-) was associated with an increased risk of ALL (OR 1.46, 95% CI 1.02-2.08 p = 0.04), B-lineage ALL (OR 1.44, 95% CI 1.00-2.08 p = 0.05) and AML (OR 2.04, 95% CI 1.03-4.03 p = 0.04) (Table 1). There was also limited evidence to suggest that the *MTHFR* 1298 variant C allele was associated with total ALL (OR 0.79, 95% CI 0.65-0.97) and B-lineage ALL (OR 0.75, 955 CI 0.61-0.93) (Table 1). When data for all polymorphisms were included in a multiple logistic regression model, similar trends were observed to those shown in Table 1 (data not shown).

When data were stratified by sex, no differences between boys and girls were observed with respect to *MTHFR* 677C>T, *SHMT1* 1420C>T, *MTR* 2756A>G, *TS* 1494del6 or *TS* 28-bp repeat polymorphisms (data not shown). However, there was some evidence that homozygosity for the *MTHFR* 1298 A>C polymorphism (CC) was associated with a decreased risk of ALL in girls (OR 0.51, 95% CI 0.30-0.89 p=0.02) and B-lineage ALL (OR 0.48, 95% CI 0.27-0.87 p=0.02), but not in boys (ALL OR 0.96, 95% CI 0.65-1.43; B-cell ALL OR 0.95, 95% CI 0.62-1.46) (data not shown).

Genotype data were also stratified according to the presence of specific chromosomal abnormalities including *MLL* and *TEL-AML1* lesions, as well as hyperdiploidy (Table 2). Homozygosity for the *MTR* 2756 polymorphism (GG) was strongly associated with *MLL* positive leukemia (OR 4.90, 95% CI 1.30-18.45 p = 0.02). Similar findings were also observed when genotypes from *MLL* positive leukemias were compared to those for all other leukemias combined (*MTR* 2756 AG OR 2.21 95% CI 1.01-4.84; *MTR* 2756 GG OR 2.60, 95% CI 0.71-9.49), and to those from cases with a normal cytogenetic profile (*MTR* 2756 AG OR 2.16, 95% CI 0.90-5.19; *MTR* 2756 GG OR 3.94, 95% CI 0.78-19.88). There was also evidence to suggest an association, although not statistically significant at the conventional 5% level, with the *TS* 28bp repeat polymorphism, 3R/3R (OR 3.53, 95% CI 0.98-12.71). Furthermore, homozygosity for the *TS* 6bp deletion polymorphism (6bp-/6bp-) was related to hyperdiploidy (OR 1.69, 95% CI 1.07-2.68 p =0.02). No significant associations were observed between TEL-AML1 positive leukemia and the polymorphisms studied with the exception of *MTHFR* 1298 where the presence of the C-allele appeared to be related to a decreased risk of TEL-AML1 positive leukemia (OR 0.52, 95% CI 0.33-0.81 p = 0.01).

When we investigated associations between the six polymorphisms we observed, as expected, associations between *MTHFR*677 and *MTHFR*1298, and between *TS* 6bp deletion and *TS* 28bp repeat polymorphisms. In addition we also detected an interaction between the *MTR* 2756 and *TS* 6bp deletion polymorphisms among ALL cases (p=0.05), such that heterozygotes for the *MTR* 2756 polymorphism were more likely to have at least one copy of the allele with the 6bpdeletion present. When we examined the effects of gene-gene interactions on leukemia risk, we observed some evidence of an interaction between *MTHFR* 1298 and *SHMT1* 1420 polymorphisms and between *MTHFR* 1298 and *TS* 28bp repeat polymorphisms for ALL (p=0.09 and p=0.11, respectively) and also B-lineage ALL (p=0.16 and p=0.07, respectively). There were too few individuals in the other subsets of cases to support this analysis.

The relationship between maternal genotype and risk of childhood leukaemia was also explored. No association between *MTHFR*677, *MTHFR*1298, or *MTR* 2756 polymorphisms and any leukemia subtype was observed in either univariate (Table 3) or multiple regression models (data not shown). Furthermore, no differences were seen when data were stratified by sex of the child or cytogenetic subtype (data not shown). When maternal and child genotype data were included in a single regression model, results were generally similar to when the child's genotype alone was considered, although for some polymorphisms the findings were more striking. For example, homozygosity for *MTR* 2756 (GG) in both mother and child was more strongly associated with *MLL* positive leukemia when compared to other leukemias (OR 8.78, 95% CI 1.92-40.13 p=0.005) and to leukemias with a normal cytogenetic profile (OR 18.75, 95% CI 1.60-220.00 p=0.02), than when the child's genotype alone was considered (all leukemias combined: *MTR* 2756 GG OR 2.60, 95% CI 0.71-9.49; normal cytogenetic profile *MTR* 2756 GG OR 3.94, 95% CI 0.78-19.88).

Discussion

We have demonstrated that the *MTR* 2756 A>G genetic polymorphism is associated with increased risk of both childhood ALL and AML, and that this risk is further increased in the subset of cases with an *MLL* chromosomal abnormality. However, in contrast to others[9-11;13;18;38], our data do not support the hypothesis that the *MTHFR* 677 C>T polymorphism modifies the risk of ALL or AML in the UK. Furthermore, no associations were observed for either *SHMT* 1420 C>T, or *TS* 28bp polymorphisms. In addition, our findings provide no evidence for a role for maternal genetic variation in folate metabolism in the etiology of childhood leukemia.

MTR encodes a vitamin B12- dependant enzyme which catalyses the remethylation of homocysteine to methionine, the precursor to S-adenosylmethionine (SAM) the universal methyl group donor [27]. The activity of MTR is dependent on vitamin B_{12} being available, as well as methionine synthase reductase (MTRR), which maintains the methionine synthase-bound B_{12} in its fully reduced active state as methyl-cob(III)alamin [39]. The MTR reaction also releases tetrahydrofolate which is re-methylated to 5,10-MeTHF for nucleotide synthesis. It has been suggested that the *MTR* 2756 polymorphism may alter enzyme activity [27] and that the G-variant could enhance the flux of one-carbon moieties available for DNA methylation processes [40]. This may provide a possible mechanism by which this polymorphism could mediate risk since hypermethylation is important in acute leukemia[41]. In addition, in a prospective cohort study investigating pregnancy complications presence of the fetal *MTR* 2756 G-allele was associated with uteroplacental insufficiency [42] suggesting it plays a part in normal fetal development, which combined with the knowledge that childhood ALL and AML originate in-utero provides further support for its role in disease aetiology. The only other study to investigate the association between *MTR* 2756 and childhood leukaemia is that by Gast et al [43], and whilst no evidence was found to support a role for *MTR*, a protective effect with polymorphisms in *MTRR* was observed.

MTHFR 677 C>T and 1298 A>C polymorphisms have been the focus of many investigations of genetic variation in the folate metabolic pathway. However, results are conflicting, with some studies reporting protective effects for *MTHFR* 677 TT [9-11;13;18;38] and 1298 CC [9-11], whilst others, including our own, have yielded little or no evidence of effect at least for 677 [12;15;17;44;45]. There are several possible reasons for these inconsistencies, one of which relates to the small case population of most previous studies. Here, however we present data on almost 1000 childhood ALLs, which is the largest single study to date. In addition, it is likely that the complexity of the folate metabolic pathway may be important as *MTHFR* is only one of over 30 enzymes involved in the pathway.

An alternative explanation, suggested in relation to colorectal cancer [46], relates to differences in circulating folate levels between populations. In the mid to late 1990s fortification of foods with folic acid became mandatory in several countries, including the USA, but not the UK, resulting in marked increases in folate intake. At the same time, recommendations were made for folate supplementation during pregnancy. Interestingly, when data from a Canadian study of 270 cases of ALL were stratified by year of birth to take account of these recommendations (pre and post 1996), protective effects of the *MTHFR* 677 T-allele and *MTHFR* 1298 C- allele were only observed in children born prior to 1996 [11]. Analogous findings have been observed for colorectal cancer where associations between polymorphisms in genes involved in the folate pathway and colorectal cancer risk appear to be modified by folate levels [47;48].

MTHFR genotypes with lower enzyme activity (677 TT and 1298 CC) favour increased availability of the non-methylated form of folate (5,10-MeTHF) for DNA synthesis and decreased levels of 5-MeTHF for DNA methylation, i.e. decreased *MTHFR* activity alters the normal intracellular distribution of folate substrates in favour of precursors for nucleotide synthesis. Thus, if adequate levels of folate are available, even if *MTHFR* activity is low, there is sufficient conversion of 5-MeTHF for DNA methylation whilst still shunting 5,10-MeTHF towards the synthesis of dUMP to dTMP and therefore preventing uracil

incorporation and chromosomal damage. This suggests that differences in folate availability may influence functional effects of *MTHFR* polymorphisms, which could possibly account for different findings between studies. In the absence of folate intake data, it is not possible to investigate this further within the UKCCS.

Folate plays an important role in embryogenesis and early fetal development via its effects on DNA methylation and synthesis[49]. As such, the well documented in utero origin of ALL has led to hypotheses that folate intake may be important in disease etiology. However, unlike for Down syndrome and neural tube defects, few studies of leukaemia have investigated maternal genotype and folate intake [8;16;19], focusing instead on the role of the child's genotype [9-13;15;17;18;38;44;45]. Our findings for mothers are, however, consistent with those reported by the only other studies to have investigated this topic, albeit on smaller populations and fewer polymorphisms [11;16].

This is the largest study to date to investigate the association between genetic variation in the folate metabolic pathway and the risk of childhood leukemia. The pathway is, however, complex and our analyses were restricted to several key enzymes and excluded other possible candidates including methionine synthase reductase as well as reduced folate carrier, which has previously been linked to risk of childhood ALL[18]. In conclusion, whilst our data do not support a role for *MTHFR* 677 C>T, they suggest that methionine synthase may be important for both ALL and AML, especially in cases with *MLL* translocations.

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Author contributions

TJL designed and secured funding for the current investigation, contributed to UKCSS genotyping and was responsible for conception of the article, interpretation of data, and producing the first draft and revisions. WTJ devised and carried out statistical analyses. DP carried out laboratory analyses of UKCCS samples and JS was responsible for data co-ordination and management. ER is responsible for UKCCS data integrity, is involved in all aspects of UKCCS design and conduct, and oversaw data acquisition and interpretation. CFS designed the original assays and carried out laboratory analysis of control samples. MTS was involved in design and conduct of the analysis of the control samples. JMA contributed to the current study design, UKCCS genotyping and pre/post WGA quality control. GMT is sample custodian for the UKCCS, secured funding for the WGA and contributed to the current study design.

All authors reviewed and revised the manuscript.

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Table 1 *MTHFR* 677C>T, *MTHFR* 1298A>C, *MTR* 2756A>G, *SHMT1* 1420C>T, *TS* 1494del6 and *TS* 28-bp repeat genotype frequencies, odds ratios (OR) and 95% confidence intervals (CI) in acute leukemia cases and controls

	Controls	Acute lymphoblastic leukemia (ALL)						Acute Myeloid Leukemia (AML)	
		Total ALL		B-lineage		T-lineage			
	n	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
*TOTAL**	824	939		765		87		89	
MTHFR (677 C>T)									
CC	359 (47.2)	374 (46.4)	1.00	302 (46.0)	1.00	37 (50.7)	1.00	47 (59.5)	1.00
CT	317 (41.7)	341 (42.4)	1.03 (0.84-1.27)	275 (41.8)	1.03 (0.83-1.29)	29 (39.7)	0.89 (0.53-1.48)	21 (26.6)	0.51 (0.30-0.87)
TT	84 (11.1)	90 (11.2)	1.03 (0.74-1.43)	80 (12.2)	1.13 (0.80-1.59)	7 (9.6)	0.81 (0.35-1.88)	11 (13.9)	1.00 (0.50-2.01)
CC v CT/TT	401 (52.8)	431 (53.6)	1.03 (0.85-1.26)	355 (54.0)	1.05 (0.85-1.30)	36 (49.3)	0.87 (0.54-1.41)	32 (40.5)	0.61 (0.38-0.98)
MTHFR (1298A>C)									
AA	350 (46.1)	408 (51.9)	1.00	343 (53.2)	1.00	33 (48.5)	1.00	41 (52.6)	1.00
AC	332 (43.7)	305 (38.8)	0.79 (0.64- 0.97)[a]	243 (37.7)	0.75 (0.60-0.93)[b]	32 (47.1)	1.02 (0.61-1.70)	26 (33.3)	0.67 (0.40-1.12)
CC	77 (10.2)	73 (9.3)	0.81 (0.57-1.15)	59 (9.1)	0.78 (0.54-1.13)	3 (4.4)	0.41 (0.12-1.38)	11 (14.1)	1.22 (0.60-2.48)
AA v AC/CC	409 (53.9)	378 (48.1)	0.79 (0.65-0.97)[c]	302 (46.8)	0.75 (0.61-0.93)[d]	35 (51.5)	0.91 (0.55-1.49)	37 (47.4)	0.77 (0.48-1.23)
SHMT1 (1420C>T)									
CC	351 (46.1)	401 (49.0)	1.00	330 (49.0)	1.00	35 (47.9)	1.00	41 (52.6)	1.00
CT	318 (41.8)	320 (39.1)	0.88 (0.71-1.09)	259 (38.5)	0.87 (0.69-1.08)	33 (45.2)	1.04 (0.63-1.71)	28 (35.9)	0.75 (0.46-1.25)
TT	92 (12.1)	97 (11.9)	0.92 (0.67-1.27)	84 (12.5)	0.97 (0.70-1.35)	5 (6.9)	0.55 (0.21-1.43)	9 (11.5)	0.84 (0.39-1.79)
CC v CT/TT	410 (53.9)	417 (51.0)	0.89 (0.73-1.09)	343 (51.0)	0.89 (0.72-1.10)	38 (52.1)	0.93 (0.57-1.50)	37 (47.4)	0.77 (0.48-1.23)
TS 28bp repeat#									
2R/2R	181 (24.0)	193 (25.4)	1.00	165 (26.6)	1.00	13 (18.1)	1.00	15 (18.5)	1.00
2R/3R	368 (48.8)	344 (45.3)	0.88 (0.68-1.13)	274 (44.2)	0.82 (0.63-1.06)	33 (45.8)	1.25 (0.64-2.43)	40 (49.4)	1.31 (0.71-2.44)
3R/3R	205 (27.2)	222 (29.3)	1.02 (0.77-1.34)	181 (29.2)	0.97 (0.72-1.3)	26 (36.1)	1.77 (0.88-3.54)	26 (32.1)	1.53 (0.79-2.98)
2R2R v others	575 (76.0)	570 (74.6)	0.93 (0.74-1.17)	457 (73.4)	0.87 (0.68-1.11)	59 (81.9)	1.43 (0.77-2.66)	66 (81.5)	1.38 (0.77-2.49)
TS (1494 del6)									
6bp+/6bp+	373 (49.0)	423 (49.5)	1.00	353 (50.4)	1.00	36 (46.8)	1.00	41 (48.8)	1.00
6bp+/6bp-	331 (43.4)	336 (39.3)	0.90 (0.73-1.10)	268 (38.3)	0.86 (0.69-1.06)	32 (41.6)	1.00 (0.61-1.65)	30 (35.7)	0.82 (0.5-1.35)
6bp-/6bp-	58 (7.6)	96 (11.2)	1.46 (1.02-2.08)[e]	79 (11.3)	1.44 (1.00-2.08)[f]	9 (11.7)	1.61 (0.74-3.51)	13 (15.5)	2.04 (1.03-4.03)[g]
6bp+/6bp+ v6bp+/6bp-/6bp-/6bp-	389 (51.0)	432 (50.5)	0.98 (0.81-1.19)	347 (49.6)	0.94 (0.77-1.16)	41 (53.2)	1.09 (0.68-1.75)	43 (51.2)	1.02 (0.64-1.58)
MTR 2756 (A>G)									
AA	510 (67.2)	531 (61.0)	1.00	431 (60.6)	1.00	48 (61.6)	1.00	43 (55.8)	1.00

AG	223 (29.4)	288 (33.1)	1.24 (1.00-1.53)[h]	240 (33.8)	1.27 (1.02-1.59)[i]	26 (33.3)	1.24 (0.75-2.05)	28 (36.4)	1.49 (0.90-2.46)
GG	26 (3.4)	51 (5.9)	1.88 (1.16-3.07)[j]	40 (5.6)	1.82 (1.09-3.03)[k]	4 (5.1)	1.63 (0.55-4.88)	6 (7.8)	2.74 (1.07-7.01)l
AA v AG/GG	249 (32.8)	339 (39.0)	1.31 (1.07-1.60)[m]	280 (39.4)	1.33 (1.07-1.65)[n]	30 (38.4)	1.28 (0.79-2.07)	34 (44.2)	1.62 (1.01-2.60)o

* Totals include individuals for whom a result was not available, and varied between SNPs

[#] includes two cases with 1R/1R; one case with 2R/4R, one case with 3R/4R; two controls with 3R/4R genotypes

[a] p = 0.03, [b] p = 0.01, [c] p = 0.02, [d] p = 0.01, [e] p = 0.03, [f] p = 0.05, [g] p = 0.04, [h] p = 0.05, [i] p = 0.03, [j] p = 0.01, [k] p = 0.02, [l] p = 0.04, [m] p= 0.01, [n] p = 0.01 , [o] p = 0.05

Table 2 Number (%) of cases and controls, odds ratios (OR) and 95% confidence intervals (CI) by leukemia subgroup for *MTHFR* 677C>T, *MTHFR* 1298A>C, *MTR* 2756A>G, *SHMT1* 1420C>T, *TS* 1494del6 and *TS* 28-bp repeat

	Controls	MLL		TEL-AML1		Hyperdiploidy	
	n (%)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
*TOTAL**	824	34		103		316	
MTHFR (677 C>T)							
CC	359 (47.2)	13 (44.8)	1.00	38 (42.2)	1.00	124 (45.9)	1.00
CT	317 (41.7)	12 (41.4)	1.05 (0.47-2.32)	36 (40.0)	1.07 (0.66-1.73)	117 (43.3)	1.07 (0.80-1.43)
TT	84 (11.1)	4 (13.8)	1.32 (0.42-4.14)	16 (17.8)	1.80 (0.96-3.38)	29 (10.8)	1.00 (0.63-1.60)
CC v CT/TT	401 (52.8)	16 (55.2)	1.10 (0.52-2.32)	52 (57.8)	1.23 (0.79-1.91)	146 (54.1)	1.05 (0.80-1.39)
MTHFR (1298A>C)							
AA	350 (46.1)	16 (55.2)	1.00	56 (62.2)	1.00	137 (50.9)	1.00
AC	332 (43.7)	8 (27.6)	0.53 (0.22-1.25)	28 (31.1)	0.53 (0.33-0.85)[a]	109 (40.5)	0.84 (0.63-1.12)
CC	77 (10.2)	5 (17.2)	1.42 (0.51-1.40)	6 (6.7)	0.49 (0.20-1.17)	23 (8.6)	0.76 (0.46-1.27)
AA v AC/CC	409 (53.9)	13 (44.8)	0.70 (0.33-1.47)	34 (37.8)	0.52 (0.33-0.81)[b]	132 (49.1)	0.82 (0.62-1.09)
SHMT1 (1420C>T)							
CC	351 (46.1)	15 (48.4)	1.00	35 (38.5)	1.00	148 (52.7)	1.00
CT	318 (41.8)	13 (41.9)	0.96 (0.45-2.04)	44 (48.3)	1.39 (0.87-2.22)	98 (34.9)	0.73 (0.54-0.98)
TT	92 (12.1)	3 (9.7)	0.76 (0.22-2.69)	12 (13.2)	1.31 (0.65-2.62)	35 (12.4)	0.90 (0.58-1.39)
CC v CT/TT	410 (53.9)	16 (51.6)	0.91 (0.45-1.87)	56 (61.5)	1.37 (0.88-2.14)	133 (47.3)	0.77 (0.58-1.01)
TS 28bp repeat#							
2R/2R	181 (23.9)	3 (10.3)	1.00	20 (24.7)	1.00	70 (26.4)	1.00
2R/3R	368 (48.7)	14 (48.3)	2.30 (0.65-8.09)	40 (49.4)	0.98 (0.56-1.73)	116 (43.8)	0.82 (0.58-1.15)
3R/3R	205 (27.1)	12 (41.4)	3.53 (0.98-12.71)	21 (25.9)	0.93 (0.49-1.77)	79 (29.8)	1.00 (0.68-1.46)
2R/2R v all others	575 (76.1)	26 (89.7)	2.73 (0.82-9.12)	61 (75.3)	0.96 (0.56-1.63)	195 (73.6)	0.88 (0.64-1.21)
TS (1494 del6)							
6bp+/6bp+	373 (49.0)	18 (54.5)	1.00	55 (56.7)	1.00	137 (47.7)	1.00
6bp+/6bp-	331 (43.4)	10 (30.3)	0.63 (0.28-1.38)	34 (35.1)	0.70 (0.44-1.10)	114 (39.7)	0.94 (0.70-1.25)
6bp-/6bp-	58 (7.6)	5 (15.2)	1.79 (0.64-5.00)	8 (8.2)	0.94 (0.42-2.06)	36 (12.6)	1.69 (1.07-2.68)[c]
6bp+/6bp+ v6bp+/6bp-/6bp-/6bp-	389 (51.0)	15 (45.5)	0.80 (0.40-1.61)	42 (43.3)	0.73 (0.48-1.12)	150 (52.3)	1.05 (0.80-1.38)
MTR 2756 (A>G)							
AA	510 (67.2)	12 (41.4)	1.00	58 (59.8)	1.00	181 (60.5)	1.00
AG	223 (29.4)	14 (48.3)	2.67 (1.21-5.86)[d]	34 (35.1)	1.34 (0.85-2.11)	101 (33.8)	1.28 (0.96-1.71)

GG	26 (3.4)	3 (10.3)	4.90 (1.30-18.45)[e]	5 (5.1)	1.69 (0.63-4.57)	17 (5.7)	1.84 (0.98-3.47)
AA v AG/GG	249 (32.8)	17 (58.6)	2.90 (1.36-6.17)[f]	39 (40.1)	1.37 (0.88-2.14)	118 (39.1)	1.34 (1.01-1.76)[g]

* Total includes cases for whom a result was not available, and varied between SNPs

[#] includes three controls with 3R/4R genotype

[a] p=0. 01, [b] p = 0.001, [c] p = 0.02, [d] p = 0.01, [e] p=0.02, [f] p=0.006, [g] p = 0.04

Table 3 The distribution of *MTHFR 677C>T, MTHFR 1298A>C* and *MTR* 2756A>G polymorphisms among mothers of children with acute leukemia.

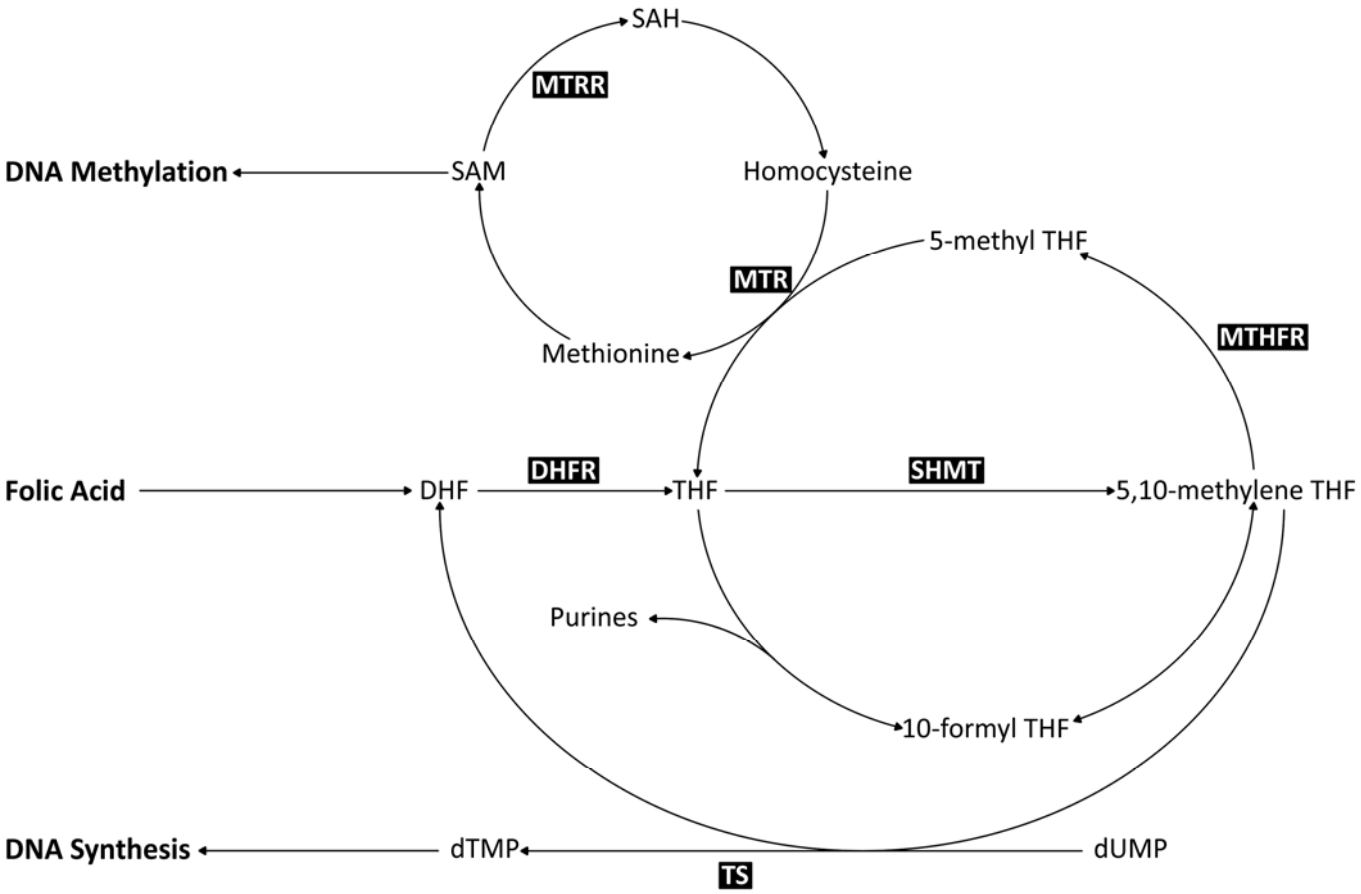
	Controls	Case mothers							
		Acute lymphoblastic leukemia (ALL)						Acute Myeloid Leukemia (AML)	
		Total ALL		B-cell ALL		T-cell ALL			
	n	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
TOTAL*	378	685		573		54		58	
MTHFR (677 C>T)									
CC	157 (44.7)	315 (47.1)	1.00	261 (46.7)	1.00	26 (49.1)	1.00	24 (45.3)	1.00
CT	159 (45.2)	277 (41.4)	0.87 (0.66-1.14)	229 (41.0)	0.87 (0.65-1.15)	21 (39.6)	0.80 (0.43-1.48)	24 (45.3)	0.99 (0.54-1.81)
TT	35 (10.0)	77 (11.5)	1.10 (0.70-1.71)	69 (12.3)	1.19 (0.75-1.86)	6 (11.3)	1.04 (0.40-2.70)	5 (9.4)	0.93 (0.33-2.62)
CC v CT/TT	194 (55.2)	354 (52.9)	0.91 (0.70-1.18)	298 (53.3)	0.92 (0.71-1.21)	27 (50.9)	0.84 (0.47-1.50)	29 (54.6)	0.98 (0.55-1.75)
MTHFR (1298A>C)									
AA	157 (44.9)	334 (50.1)	1.00	285 (51.2)	1.00	27 (50.9)	1.00	24 (46.2)	1.00
AC	151 (43.1)	254 (38.1)	0.79 (0.60-1.04)	205 (36.9)	0.75 (0.56-1.00)	24 (45.3)	0.92 (0.51-1.67)	18 (34.6)	0.78 (0.41-1.49)
CC	42 (12.0)	78 (11.8)	0.87 (0.57-1.33)	66 (11.9)	0.87 (0.56-1.34)	2 (3.8)	0.28 (0.06-1.21)	10 (19.2)	1.56 (0.69-3.51)
AA v AC/CC	193 (55.1)	332 (49.9)	0.81 (0.62-1.05)	231 (48.8)	0.77 (0.59-1.01)	26 (49.1)	0.78 (0.44-1.40)	28 (53.8)	0.95 (0.53-1.70)
MTR 2756 (A>G)									
AA	239 (68.3)	429 (65.7)	1.00	358 (64.4)	1.00	35 (66.0)	1.00	31 (57.4)	1.00
AG	97 (27.7)	201 (31.2)	1.15 (0.86-2.50)	168 (30.2)	1.16 (0.86-1.56)	16 (30.2)	1.13 (0.60-2.13)	21 (38.9)	1.67 (0.91-3.05)
GG	14 (4.0)	33 (5.1)	1.31 (0.69-2.50)	30 (5.4)	1.43 (0.74-2.75)	2 (3.8)	0.98 (0.21-4.48)	2 (3.7)	1.10 (0.24-5.08)
AA v AG/GG	111 (31.7)	234 (36.3)	1.17 (0.89-1.55)	198 (35.6)	1.19 (0.90-1.58)	18 (34.0)	1.11 (0.60-2.04)	23 (42.6)	1.60 (0.89-2.87)

* Totals include individuals for whom a result was not available, and varied between SNPs

OR – odds ratio

CI – confidence interval

Figure 1 Metabolic folate pathway



Metabolites: 5-methylTHF, 5-methyltetrahydrofolate; 10-formylTHF, 10-formyltetrahydrofolate; SAM- adenosylmethionine; SAH, S-adenosylhomocysteine; DHF, dihydrofolate; THF, tetrahydrofolate; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate

Enzymes: MTR, methionine synthase; SHMT, serine hydroxymethyltransferase; MTHFR, 5,10-methylenetetrahydrofolate reductase; TS, thymidylate synthase