Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia

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Low folate intake as well as alterations in folate metabolism as a result of polymorphisms in the enzyme methylenetetrahydrofolate reductase (*MTHFR*) have been associated with an increased incidence of neural tube defects, vascular disease, and some cancers. Polymorphic variants of *MTHFR* lead to enhanced thymidine pools and better quality DNA synthesis that could afford some protection from the development of leukemias, particularly those with translocations. We now report associations of *MTHFR* polymorphisms in three subgroups of pediatric leukemias: infant lymphoblastic or myeloblastic leukemias with *MLL* rearrangements and childhood lymphoblastic leukemias with either *TEL-AML1* fusions or hyperdiploid karyotypes. Pediatric leukemia patients ($n = 253$ total) and healthy newborn controls ($n = 200$) were genotyped for *MTHFR* polymorphisms at nucleotides 677 (C→T) and 1,298 (A→C). A significant association for carriers of *C677T* was demonstrated for leukemias with *MLL* translocations (*MLL*+, $n = 37$) when compared with controls [adjusted odd ratios (OR) = 0.36 with a 95% confidence interval (CI) of 0.15–0.85; $P = 0.017$]. This protective effect was not evident for *A1298C* alleles (OR = 1.14). In contrast, associations for *A1298C* homozygotes (CC; OR = 0.26 with a 95% CI of 0.07–0.81) and *C677T* homozygotes (TT; OR = 0.49 with a 95% CI of 0.20–1.17) were observed for hyperdiploid leukemias ($n = 138$). No significant associations were evident for either polymorphism with *TEL-AML1*+ leukemias ($n = 78$). These differences in allelic associations may point to discrete attributes of the two alleles in their ability to alter folate and one-carbon metabolite pools and impact after DNA synthesis and methylation pathways, but should be viewed cautiously pending larger follow-up studies. The data provide evidence that molecularly defined subgroups of pediatric leukemias have different etiologies and also suggest a role of folate in the development of childhood leukemia.

Pediatric acute leukemia represents a group of diseases without evidence for highly penetrant germ-line-inherited susceptibility except for cases with rare genetic instability syndromes or immunodeficiency thought to be involved in approximately 5% of all cases. The causes of the majority of pediatric acute leukemias are unknown and likely to involve an interaction between the environment, hematopoietic development, weak susceptibility loci within an individual's genetic constitution, and chance. The diversity of biological and molecular subtypes of pediatric leukemias suggests that different etiological factors, and perhaps different inherited susceptibility, may play a role in specific subtypes (1). An example is the case of infant leukemias with *MLL* translocations, in which molecular and epidemiologic evidence implicate the activity of topoisomerase-II-inhibiting compounds in causation (2, 3). The influence of gene-environment interaction was suggested in an association of a null variant of a detoxifying enzyme, *NAD(P)H:quinone oxidoreductase* (*NQO1*), with infant leukemias that have *MLL* translocations (*MLL*+ leukemia; ref. 4). This association seemed specific for *MLL*-rearranged leukemias and was not apparent in other more common molecular subtypes of pediatric leukemias (those with *TEL-AML1* fusions and hyperdiploidy [i.e., >50 chromosomes]).

The role of vitamin-dependent one-carbon (methyl group) metabolism has come under intense scrutiny in recent years leading to the discovery that disruption of homeostasis in the one-carbon pool affects risks of heart disease, neural tube defects, and cancer. Such disruption can occur in the presence of deficiencies in the two essential micronutrients involved in this metabolism: folate and cobalamin (vitamin B_{12}). Alterations in metabolism may occur also with genetic variation at any of the more than two dozen enzymes directly involved in maintaining homeostasis of the one-carbon pool, several of which are known to be polymorphic. One-carbon metabolism is divided into two main branches: one branch consists of reactions involving purine and thymidine synthesis, and the other branch involving synthesis of methionine and *S*-adenosylmethionine (AdoMet) for protein and polyamine synthesis and methylation reactions (Fig. 1 *Right* and *Left*, respectively). An enzyme that shunts methyl groups from the first of these branches to the second is MTHFR. MTHFR irreversibly converts 5,10-methylenetetrahydrofolate (5,10-CH_2-THF) to 5-methyl-THF, which then donates a methyl group to homocysteine to produce methionine (Fig. 1). Individuals who are severely deficient in MTHFR activity because of a germline mutation have excessive amounts of homocysteine in the blood and urine and develop severe mental retardation and thrombo-occlusive vascular disease (5). Interestingly there exist two common low-function polymorphic variants of *MTHFR*: the T variant at nucleotide 677 (*MTHFR C677T*) and the C variant at nucleotide 1298 (*MTHFR A1298C*). The first of these variants, *C677T*, has been shown to be associated with higher baseline homocysteine levels in the serum and is associated with in-

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Abbreviations: NQO1, NAD(P)H/quinone oxidoreductase; AdoMet, *S*-adenosylmethionine; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate; C677T: the T nucleotide variant at nucleotide 677 in MTHFR; A1298C, the C variant at nucleotide 1,298 in MTHFR; ALL, acute lymphocytic leukemia; OR, odds ratio; CI, confidence interval.

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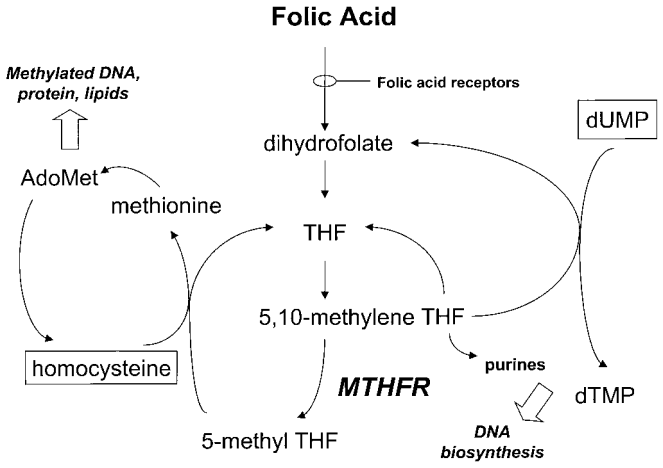

Fig. 1. Overview of folic acid metabolism and the role of methylenetetrahydrofolate reductase (MTHFR). Critical metabolites with putative associations to disease are noted in boxes. Homocysteine accumulation is associated with cardiovascular and neural tube malformations; deoxyuridine monophosphate accumulation is associated with cancer. AdoMet, *S*-adenosylmethionine; THF, tetrahydrofolate; dUMP, deoxyuridine monophosphate; dTMP, deoxythymidine monophosphate.

creased risk of vascular disease and neural tube defects (6). This polymorphism is linked paradoxically to a lower risk of colon cancer (7–9). This lower risk is thought to be caused by the increased fidelity of DNA synthesis afforded by the greater availability of the MTHFR substrate 5,10-CH_2-THF for DNA synthesis, in particular the increased availability of methyl groups for conversion of uracil to thymidine. Inadequate thymidine pools result in increased incorporation of uracil into DNA resulting in strand breaks (10), which are the precursors for chromosome translocations and deletions.

Low-function variants of *MTHFR* have been reported to be protective for adult acute lymphocytic leukemia (ALL; ref. 11). For pediatric leukemia, we hypothesized that this protection should be more pronounced in subtypes characterized by chromosomal translocations, because the formation of translocations in these leukemias are thought to involve DNA double-strand break formation by means other than aberrant V(D)J recombinase activity (12, 13). Both the most common translocations in infant leukemia (*MLL-AF4*) and in common childhood ALL (*TEL-AML1*) occur *in utero* in most if not all patients (14–16). Pregnancy is a time of extreme folate requirement characterized by the incidence of folate-responsive megaloblastic anemia in 24% of nonsupplemented pregnancies in undeveloped countries and 2.5–5% of pregnancies in the developed world (17). This form of anemia, as well as subtler biochemical and hematological signs of deficiency, are caused ultimately when the synthesis of thymidine is reduced beyond a critical level. Given the common *in utero* origin of childhood leukemia translocations and the high demands for folate, we predicted that *MTHFR* low-function alleles would confer protection for pediatric leukemia with translocations in a similar if not more pronounced fashion, because these alleles protect against leukemia in adults. Subgroups of leukemia including those with *MLL* fusions and *TEL-AML1* fusions, as well as those with hyperdiploidy (>50 chromosomes in diagnostic karyotypes) were genotyped for *MTHFR* in comparison with normal newborn controls.

Materials and Methods

Patients and Control Samples. Between 1992 and 1998, the United Kingdom Childhood Cancer Study attempted to enroll all new patients with childhood leukemia in the U.K. aged <15 years into a study testing five etiological hypotheses (18). Patient samples taken at the time of diagnosis were screened and classified for common molecular subgroups of pediatric leukemia by banded karyotyping and fluorescence *in situ* hybridization (for hyperdiploidy), banded karyotyping and reverse transcription (RT) –PCR (for *MLL* fusions), and RT-PCR for *TEL-AML1* fusions (see ref. 18). Blood samples from patients at the time of remission were stored also; aliquots of these remission samples were used for genotyping in the present study except for 16 *MLL*+ samples in which remission DNA was not available and diagnostic DNA was used instead. For the cases of common ALL (cALL), available remission DNA samples were used and not diagnostic DNA. Given that virtually all cALL patients enter a first remission, selection of patients is not biased by this parameter. Controls consisted of umbilical cord blood samples obtained from unselected healthy newborn infants in the Manchester, U.K. area and were all U.K. whites. A small proportion (no greater than 14%) of cases was from minority ethnic groups in the U.K. (i.e., U.K. Indian Asians, U.K. blacks, and U.K. East Asians). No attempt was made to screen out such individuals, but we have no evidence that they are represented disproportionately in any leukemia subgroup. Ethnic heterogeneity among cases was not eliminated in these analyses because of ambiguities in classification of some individuals as well as some admixture. Enrolled in this study were 37 *MLL*-rearranged infant leukemias, 78 *TEL-AML1*+ leukemias, 138 hyperdiploid leukemias, and 200 cord blood controls.

MTHFR Genotyping. Standard PCR/restriction fragment length polymorphism analysis was used for genotyping. The regions containing the two polymorphisms were amplified separately. For the nucleotide 677 polymorphism, the primers CCTTGAACAGGTGGAGGCC (intronic) and CAAAGAAAAGCTGCGTGATGAT (exonic) were used (158-bp product), and for the nucleotide 1,298, the primers GCAAGTCCCCCAAGGAGG and GGTCCCCACTTCCAGCATC were used (145-bp product). PCR reactions (50 μl) contained 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 2.5 pmol of each dNTP, 1.25 units of *Taq* polymerase, and 80 ng of DNA and were cycled 40 times (1 min at 94°C, 1 min at 60°C, 1 min at 72°C). Amplification success was monitored by agarose electrophoresis. The remainder of the PCR reaction was subjected to digestion with *Hin*F1 (for nucleotide 677) or *Mbo*II (for nucleotide 1,298) and subjected to electrophoresis in 0.7% agarose with 2% Synergel (Diversified Biotech). For the case of nucleotide 677, an undigested PCR product (158 bp) indicated a homozygous wild type, three bands of 158, 130, and 28 bp indicated a heterozygous, and two fragments of 130 and 28 bp indicated a homozygous variant individual. For the case of nucleotide 1,298, three fragments of 29, 37, and 79 bp indicated wild type, four fragments of 29, 37, 79, and 108 bp indicated heterozygous, and two fragments of 37 and 108 bp indicated homozygous variant individuals.

Statistical Analysis. Analyses used a combination of logistic regression (19) and exact methods (20) and were implemented in EGRET (21). Odds ratios (OR) and 95% confidence intervals (CI) were calculated. All *P* values are two-sided, and where logistic regression is applied, are based on the maximum likelihood test. Adjusted estimates of the ORs are derived by multivariate analyses and, when using exact methods, with stratification of genotypes at one locus by genotypes at the other. Formal testing of statistical interaction was based on logistic regression modeling and on exact tests for equality of the ORs for one genotypic variant across strata defined by genotypes at the other locus. Exact methods were considered preferable whenever expected numbers in any cell are less than five and for

Table 1. Allele frequencies of *C677T* and *A1298C* in study population

Classification, *n*	*C677T*, %	*A1298C*, %
Control, 200	35.8	32.4
MLL, 37	21.6	40.5
TEL-AML1+, 78	38.5	24.0
Hyperdiploid, 138	30.8	28.1

consistency, most results reported here are based on exact methods.

Results

Genotyping was successful for both polymorphisms on all individuals except for five *TEL-AML1*+ and one hyperdiploid leukemia that did not amplify for the nucleotide 1,298 polymorphism. These cases were excluded for analysis concerning the nucleotide 1,298 polymorphism and the multivariate analyses.

Control samples demonstrated allele frequencies of 35.8% for *C677T* and 32.4% for *A1298C* (Table 1), which are close to the range of 33–37% allele frequency found for these variants in other studies of European whites (11, 22–24). The allele frequencies of the polymorphisms in the leukemia subgroups varied in both directions from the controls (Table 1). *MLL*+ leukemias had a lower frequency of *C677T* alleles but a higher frequency of *A1298C*. *TEL-AML1* leukemias demonstrated the opposite profile, whereas hyperdiploid leukemias had lower allele frequencies than controls at both loci. The inclusion of no greater than 14% of minority ethnic groups among the cases may bias the genotype frequency downward for *MTHFR C677T*. U.K. Indian Asian have an allele frequency of 15% (25) and Sub-Saharan Africans of 6% (22), whereas East Asians have an allele frequency similar to European whites at 35–40% (22, 26). In a large U.K. study of childhood cancer and ethnic groups, similar relative frequencies of leukemia (including the subgroups common ALL and B cell leukemia) were observed among U.K. whites, U.K. Indian Asians, and U.K. blacks (27). Assuming similar rates in the current study, the overall allele frequency of the controls should decrease a minor 3% had the controls been matched ethnically. An adjustment of ethnicity or the exclusion of minorities in the analysis in the case-control comparisons would not change any of the significant results to nonsignificance (data not shown). Frequencies of the nucleotide 1,298 polymorphism in various ethnic groups are not known; however, our major results for this polymorphism are based on excess of CC-homozygous genotypes rather than allele frequency.

Statistical analyses were performed on each polymorphism individually (Table 2) and on each polymorphism after adjusting for the other (multivariate analysis, Table 3). Analyses are shown

Table 3. Multivariate analyses of individual MTHFR polymorphisms, controlled for the other, in molecular cytogenetic subgroups of the U.K. Childhood Cancer Study

	MLL vs. controls	*TEL-AML1* vs. controls	Hyperdiploid vs. controls
*C677T**			
CC	1.00†	1.00†	1.00†‡
CT	0.29 (0.09–0.79)	1.09 (0.56–2.12)	0.82 (0.49–1.38)‡
TT	0.67 (0.16–2.53)	0.91 (0.33–2.44)	0.49 (0.20–1.17)‡
P value	0.032	0.91	0.18
CT + TT§	0.36 (0.15–0.85)	1.03 (0.55–1.92)	0.82 (0.51–1.34)
P value¶	0.017	1.0	0.47
*A1298C**			
AA	1.00†	1.00†	1.00†
AC	1.35 (0.53–3.63)	0.58 (0.30–1.13)	0.98 (0.58–1.64)
CC	1.33 (0.32–5.29)	0.56 (0.16–1.76)	0.26 (0.07–0.81)
P value	0.82	0.17	0.028
AC + CC§	1.14 (0.49–2.73)	0.59 (0.31–1.08)	0.92 (0.57–1.49)
P value¶	0.90	0.09	0.80

*OR for each variant adjusted by the other variant (95% confidence limits derived using exact methods). Exact *P* values for ORs for one variant being different across strata of the other all exceeded 0.7 (ie, no evidence of statistical interaction).
†Reference group.
‡Test for trend from CC to CT and TT, $P = 0.075$.
§Combined heterozygous and homozygous ''variant'' genotypes compared to homozygous ''wild type'' as reference.
¶Adjusted *P* value for heterogeneity by levels of the variant (maximum likelihood test).

Table 2. Univariate analyses of MTHFR polymorphisms in molecular cytogenetic subgroups of the United Kingdom Childhood Cancer Study

	Leukemia: molecular subgroups N, %			Controls	*MLL* vs. controls	*TEL-AML1* vs. controls	Hyperdiploid vs. controls
Variant	*MLL*+	*TEL-AML1*+	Hyperdiploid	N, %	OR, 95% CI	OR, 95% CI	OR, 95% CI
C677T							
CC	26 (70%)	31 (40%)	67 (49%)	89 (44.5%)	1.00*	1.00*	1.00*
CT	6 (16%)	34 (44%)	57 (41%)	79 (39.5%)	0.26 (0.10–0.66)	1.24 (0.70–2.19)	0.96 (0.60–1.53)
TT	5 (14%)	13 (17%)	14 (10%)	32 (16%)	0.53 (0.19–1.51)	1.17 (0.54–2.50)	0.58 (0.29–1.17)
p (heterogeneity)†					0.008	0.76	0.29
CT + TT‡	11 (30%)	47 (60%)	71 (51%)	111 (55.5%)	0.34 (0.16–0.72)	1.22 (0.71–2.07)	0.85 (0.55–1.31)
P (heterogeneity)§					0.004	0.47	0.46
A1298C							
AA	13 (35%)	44 (60%)	65 (47%)	93 (47%)	1.00*	1.00*	1.00*
AC	18 (49%)	23 (32%)	67 (49%)	83 (42%)	1.55 (0.72–3.36)	0.59 (0.33–1.05)	1.16 (0.74–1.21)
CC	6 (16%)	6 (8%)	5 (4%)	23 (12%)	1.89 (0.64–5.44)	0.55 (0.21–1.45)	0.31 (0.11–0.86)
p (heterogeneity)†					0.40	0.14	0.02
AC + CC‡	24 (65%)	29 (40%)	72 (53%)	106 (53%)	1.62 (0.78–3.36)	0.58 (0.34–1.00)	0.97 (0.61–1.50)
P (heterogeneity)§					0.19	0.047	0.90

*Reference group.
†*P* value for heterogeneity across three categories.
‡Combined heterozygous and homozygous ''variant'' genotypes compared to homozygous ''wild type'' as reference.
§*P* value for heterogeneity across two categories.

for both heterozygotes and homozygotes at each polymorphism individually and with the two combined into one group of "low-function" genotypes (Tables 2 and 3). A significant protective affect of the combined genotypes (CT + TT) at nucleotide 677 was shown for *MLL+* leukemias (OR = 0.34 with a 95% CI of 0.16–0.72, $P = 0.004$; Table 2); this effect was scarcely altered when controlled for nucleotide 1,298 (OR = 0.36 with a 95% CI of 0.15–0.85; Table 3). When nucleotide 677 CT and TT were considered separately, the protective effect seemed stronger for the heterozygote CT (OR = 0.26 with a 95% CI of 0.10–0.66; Table 2) although still evident for homozygote TT (OR = 0.53 with a 95% CI of 0.19–1.51). These OR estimates on individual genotypes are imprecise because of the small number of individuals in each group (six and five for CT and TT genotypes, respectively). In contrast, for the *MLL+* leukemias, there was no evidence of a protective effect for the *A1298C* variant (AC + CC, OR = 1.62 with a 95% CI of 0.78–3.36; Table 2). An OR of greater than one indicates that the variant is an "at risk" allele; however, it must be noted that the two alleles are in tight negative linkage disequilibrium (28). After adjusting for the nucleotide 677, the *A1298C* OR falls close to unity (OR = 1.14 with a 95% CI of 0.49–2.73). There were no differences between genotypes at either nucleotide 677 ($P = 0.45$, Fisher's exact test) or nucleotide 1,298 ($P = 1$) between the DNA samples obtained at diagnosis ($n = 16$) and remission ($n = 21$), indicating no observed effect of *MTHFR* genotype on likelihood of remission and hence no detectable selection bias among these groups.

Although *TEL-AML1* leukemias represent a translocation subgroup like the *MLL+* group, there was very little evidence of association of these cases with either polymorphism. A marginally significant association was apparent for *TEL-AML1* leukemias, in which combined AC + CC genotypes at nucleotide 1,298 were underrepresented in leukemias vs. controls (OR = 0.58 with a CI of 0.34–1.00, $P = 0.05$; Table 2).

A significant protective effect of the *A1298C* variant in its homozygous form was apparent for hyperdiploid leukemias (OR = 0.31 with a 95% CI of 0.11–0.86; Table 2), which was strengthened after adjusting for nucleotide 677 (OR = 0.26 with a 95% CI of 0.07–0.81; Table 3). This association was not apparent in heterozygotes or with the combination of *MTHFR 1298* AC with CC (OR = 0.98; Table 3). There was some evidence that *C677T* was protective for hyperdiploid leukemia; the test for trend (across CC, CT, TT) approached statistical significance in the multivariate analysis adjusting for genotype at nucleotide 1,298. In tests for statistical interaction, all *P* values for the multivariate analyses exceeded 0.5. Therefore, there is no evidence of statistical interaction between the two polymorphisms in any of the three leukemia categories.

Discussion

Interest in folate and folate metabolism is high because of the vast array of cellular reactions involving one-carbon units and the wide variety of disease states resulting from deficiencies in folate and cobalamin (vitamin B_{12}; ref. 6). Even without overt disease, modest perturbation of folate levels or metabolism is thought to increase risk for vascular diseases and cancer. The role of folate in cancer probably is due to defects in different but related branches of folate metabolism including defective cell division caused by a shortage of thymidine for DNA synthesis and a shortage of methyl groups for DNA methylation. These pathways have been linked to chromosome instability in both *in vitro* and human studies of both cancerous and normal tissues (29–32).

A central enzyme in folate metabolism is 5,10-methylenetetrahydrofolate reductase (MTHFR), which shunts methyl groups from DNA synthesis to methylation pathways with the concomitant conversion of homocysteine to methionine (Fig. 1). The low-function variant *C677T* causes an accumulation of homocysteine and is associated with increased risk of vascular diseases, especially in individuals under low-folate stress. However, this same variant conserves folate within a cyclic pathway inside the cell by shunting one-carbon groups toward thymidine and purine synthesis, which may explain its association with the reported lower risk for colorectal carcinoma and leukemia. The *A1298C* polymorphism is less well characterized but is reported also to be associated with a lower risk of leukemia development in adults (11).

Starting with the hypothesis that different molecular subtypes of leukemia will have different etiologies, we began with the three most distinctive molecular subgroups of pediatric leukemia. Very few studies have examined inherited susceptibility to molecularly defined subtypes of childhood leukemia, and of these, all have been restricted to infant leukemia. We acknowledge that our series, although large in terms of molecular subgroups, is nevertheless small in epidemiological terms. We cannot exclude chance as an explanation for our positive results nor lack of statistical power as explanation for our negative results.

The best characterized subgroup is infant leukemia with *MLL* translocations. These leukemias (AML and ALL) are known to be initiated *in utero* and have been linked epidemiologically and molecularly to the involvement of topoisomerase II-inhibiting dietary and/or environmental chemicals (2, 3, 33–35). In addition, this subgroup has been associated with a low-function variant of the *NAD(P)H:quinone oxidoreductase* gene, implicating the involvement of quinone-containing topoisomerase II-inhibiting agents such as benzene and flavonoid metabolites (4). We report here that this subgroup also has a lower frequency of *C677T* alleles compared with controls (OR = 0.36 with a 95% CI of 0.15–0.85). The *C677T* polymorphism conserves intracellular folate for DNA synthesis pathways (10); conservation of folate for DNA synthesis would slow the incorporation of uracil into DNA, a process that may protect against strand breaks and *MLL* translocations. Our data are consistent with the hypothesis that *C677T* may protect against the development of leukemia with *MLL* translocations.

The *MLL+* patients and controls reported here also were genotyped for *NQO1* alleles as previously reported (4). After adjusting for *NQO1* genotypes (*NQO1 C609T* heterozygotes and homozygotes), the OR for *MTHFR C677T* was changed only slightly (OR = 0.41 vs. 0.36 without adjustment for *NQO1*; Table 3 and data not shown), suggesting that polymorphisms at these two genes are independent modifiers of risk for *MLL+* leukemia. However, because of the small number of *MLL+* patients in the current study, these results should be viewed with caution. Future studies of potential gene–environment interaction in the etiology of infant leukemia should consider polymorphisms in these genes with careful assessment of the appropriate environmental stresses (i.e., diet and chemical exposure) that would interact in individuals with at-risk alleles of both genes.

The other two molecular subgroups in this study, *TEL-AML1+* and hyperdiploid leukemias, are similar to each other in cell type (pre-B cell, common ALL), age of distribution, and prognosis (1). The *TEL-AML1* molecular lesion is known to occur predominantly *in utero* (16) and has genomic sequence features, suggesting that it arises from nonhomologous recombination and error-prone repair following double-strand breaks (13). Hyperdiploid ALL could have a similar origin, but direct evidence for this is currently unavailable. The *TEL-AML1* translocation itself is not sufficient for leukemia but requires a second postnatal "hit" to induce overt leukemia (1). This event most often seems to involve the deletion of the second *TEL* allele (36) and can occur more than a decade after the occurrence of the translocation *in utero* (37). The current study provides little evidence for any *MTHFR* allelic association with *TEL-AML1*

leukemia. However, low statistical power from the small number analyzed means that a modest effect cannot be excluded.

Hyperdiploid leukemias are characterized by a change in chromosome number, which are presumed to arise via mitotic nondisjunction rather than DNA strand breaks or translocations. We had not predicted an association between inherited *MTHFR* alleles and hyperdiploid leukemias. However, to our surprise these leukemias demonstrated significant associations. The most significant result was for the *A1298C* CC homozygotes, which were 4-fold less frequent as homozygote AA individuals in the hyperdiploid cases compared with controls (OR = 0.26 with a 95% CI of 0.07–0.81). An association was suggested also for *C677T* ($P = 0.075$, test for trend across TT, CT, CC). Given the lower phenotypic effect of *A1298C* on MTHFR activity *in vitro* and the complete lack of effect of this polymorphism on activity in yeast-expressed MTHFR (38), it seems counterintuitive that this is the most strongly associated allele. However, there is some precedent for the stronger association of *A1298C* than *C677T*, being provided by a study of *MTHFR* alleles in adult ALL (11). We speculate that the effect of *A1298C* in the pathway toward hyperdiploid leukemias is a result of perturbation of specific attributes of one-carbon metabolism rather than simply a generalized repression of MTHFR activity. We emphasize that this result could be attributable to chance, especially because no prior hypothesis was involved.

One-carbon methylation pathways pass through AdoMet, which is the cell's primary methyl donor. *MTHFR* nucleotide 1,298 is located outside of the catalytic domain and on the AdoMet-regulatory domain of the enzyme (39, 40), where it may be involved in protein stabilization (38). It is possible that *A1298C* polymorphism can in some fashion alter one-carbon metabolism within a cell, potentially under conditions of folate stress such as pregnancy, to prevent aberrant methylation patterns that would lead to chromosome instability. Aberrant methylation patterns are known to affect chromosome stability. Hypomethylation was associated with chromosome instability in a number of studies, e.g., see refs. 32, 41, and 42. Chromosome instability is related also to constitutive genetic defects in DNA methylation (42, 43) as well as methylation abnormalities developed during carcinogenesis (29, 44). Future studies will have to tease out whether effects of *MTHFR A1298C* occur pre- or postnatally in children who develop hyperdiploid leukemias, and whether this putative pathway is preventable with supplementary dietary folate.

One further speculation prompted by these data concerns the frequency of the particular chromosome involvement in hyperdiploid ALL. The most frequent chromosome with one or two extra copies is chromosome 21. No fewer than 97% of hyperdiploid leukemias have extra copies of chromosome 21 (45), and chromosome 21 is the only chromosome that occurs as a sole (nonconstitutive) trisomy in ALL (46). This chromosome harbors the reduced folate carrier gene (RFC), which is important for the cells' uptake of folate from serum. When cells are stressed for folate under *in vitro* culture conditions, they respond by amplifying or up-regulating the RFC gene in an attempt to sequester folate from the serum (47, 48). This sort of mechanism would be critical especially for individuals with variant *MTHFR* alleles who are known to have lower serum folate. It is possible therefore that the gain of an extra chromosome 21, perhaps generated randomly, would be maintained with selective advantage (in relation to concomitant nondisjunction of other chromosomes) because of the gene dosage effect of added RFC expression. Additional copies of chromosome 21 will be associated with additional RFC expression and greater folate uptake that therefore provides a plausible functional explanation for the preferential involvement of chromosome 21 in pediatric ALL (49, 50). Additionally, leukemias with hyperdiploidy including trisomy chromosome 21 are highly responsive clinically (and curable) accumulating high levels of the folate analog, methotrexate (51).

Over 80% of whites and East Asians carry an *MTHFR* variant, and 40% of people carry two. This means that 40% of the population carries two variant allelic copies of *MTHFR* (because the alleles are in negative linkage disequilibrium—i.e., the alleles are inherited on separate chromosomes) rendering the concept of a "wild-type" *MTHFR* individual irrelevant. This high frequency of *MTHFR* polymorphisms combined with the apparent associations with childhood and adult leukemia provide evidence that the attributable risk of childhood leukemia caused by altered features of one-carbon metabolism involving folate may be quite high. Future studies would need to confirm the associations found in this study and might consider *MTHFR* and other one-carbon enzyme polymorphisms combined with an assessment of total folate intake at appropriate times to estimate the gene–environment interaction in the etiology of leukemia.

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