



HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

5,10-Methylenetetrahydrofolate Reductase Polymorphisms and Leukemia Risk: A HuGE Minireview

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Received for publication July 12, 2002; accepted for publication October 16, 2002.

Leukemias commonly arise as a result of DNA translocations, inversions, or deletions in genes regulating blood cell development or homeostasis. Folate deficiency has been associated with uracil misincorporation into DNA and DNA double strand breaks during uracil excision repair, thus increasing the risk of chromosomal aberrations. Methylenetetrahydrofolate reductase (MTHFR) directs 5,10-methylenetetrahydrofolate toward methionine synthesis at the expense of DNA synthesis. Two *MTHFR* polymorphisms, *C677T* and *A1298C*, have been associated with reduced enzyme activity and *C677T* with altered distribution of intracellular folate metabolites. Rapidly replicating cell types, such as hematopoietic cells, may be especially sensitive to changes in the availability of intracellular folate. Three case-control studies have evaluated the association between *MTHFR* polymorphisms and the risk of acute leukemia, and they suggest that both adults and children with the variant forms of *MTHFR* have a decreased risk of lymphoid leukemias. However, no modification in risk has been observed for myeloid leukemias, suggesting that differences in folate requirements or susceptibility to chromosomal damage may exist between myeloid and lymphoid cells. Further investigation into the association between *MTHFR* polymorphisms and the risk of leukemia is warranted. It should include larger sample sizes and other polymorphisms in folate metabolism and address interactions with folate status.

A1298C; *C677T*; epidemiology; genetics; leukemia; *MTHFR*; neoplasms; polymorphism (genetics)

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; bp, base pair(s); CI, confidence interval; CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; cSHMT, cytosolic serine hydroxymethyltransferase; FAD, flavin adenine dinucleotide; kb, kilobases; MLL, myeloid/lymphoid or mixed lineage leukemia; MS, methionine synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase; OR, odds ratio; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase.

Editor's note: This article is also available on the website of the Human Genome Epidemiology Network (<http://www.cdc.gov/genomics/hugenet/default.htm>).

GENE AND GENE PRODUCT

The primary function of folate is as a carrier for single carbon fragments in the conversion of homocysteine to methio-

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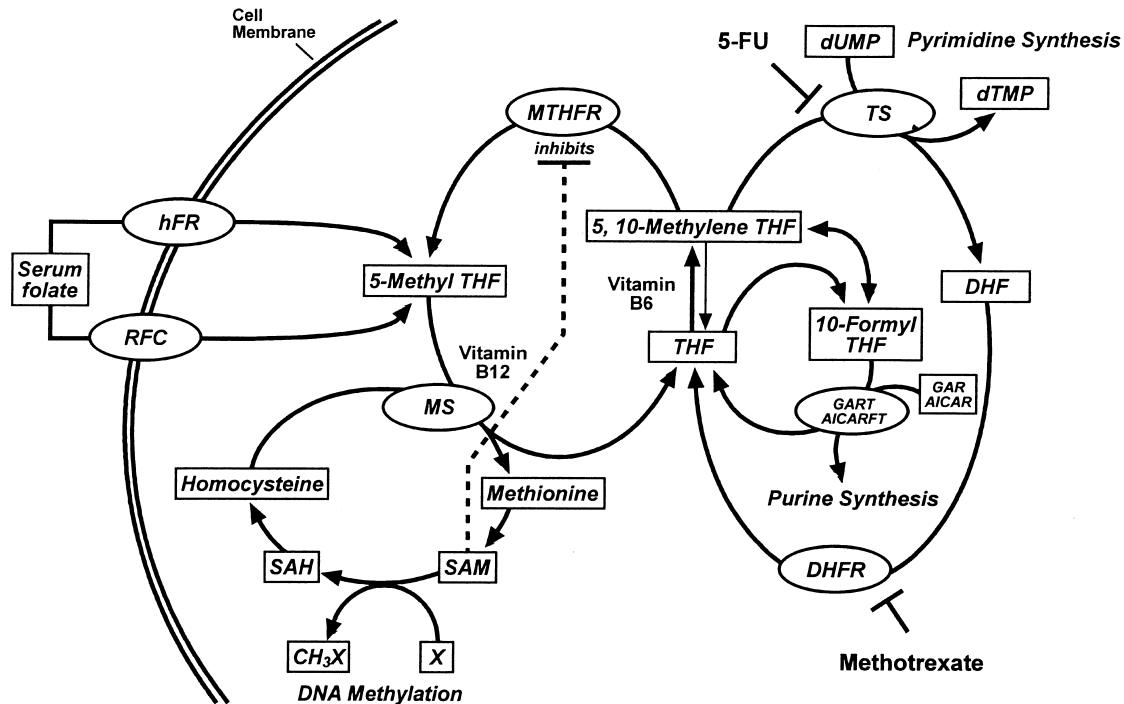


FIGURE 1. Folate metabolism, links to DNA synthesis and methylation, and targets of major antifolate agents (simplified). Key enzymes are denoted as ovals, and substrates are denoted as rectangles. AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; AICARFT, 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; CH_3 , methyl group; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; 5-FU, 5-fluorouracil; GAR, glycinamide ribonucleotide; GART, glycinamide ribonucleotide transformylase; hFR, human folate receptor; MS, methionine synthase; MTHFR, 5,10-methylenetetrahydrofolate reductase; RFC, reduced folate carrier; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate; TS, thymidylate synthase; X, a variety of substrates for methylation. (Reprinted with permission from *Pharmacogenomics* (96). Copyright 2002, Ashley Publications, Ltd.)

nine and in purine and pyrimidine synthesis. Figure 1 summarizes key enzymes of intracellular folate metabolism. The enzyme 5,10-methylenetetrahydrofolate (MTHFR) catalyzes the irreversible reduction of 5,10-methylene tetrahydrofolate (THF) to 5-methyl-THF, the primary form of serum folate. MTHFR has been described as being located at the branch point of directing folate metabolites toward remethylation of homocysteine and away from DNA and RNA biosynthesis (1). The gene for the MTHFR enzyme (EC 1.5.1.20) is found at the end of the short arm of chromosome 1 (1p36.3) (2). The complementary DNA sequence of this gene is approximately 2.2 kilobases (kb), which includes 11 exons ranging in size from 103 base pairs (bp) to 432 bp (2, 3).

GENE VARIANTS

There are two well-described, commonly occurring polymorphisms in the *MTHFR* gene: *C677T* and *A1298C*. Other polymorphisms have been reported at bp 1059, bp 1289, bp 1317, and bp 1793 (4–6). These polymorphisms are less common than the *C677T* or *A1298C* polymorphism, and their functional relevance has not yet been investigated.

C677T polymorphism

The *C677T* polymorphism occurs in exon 4 and results in an alanine-to-valine substitution at codon 222. The polymorphism lies at the base of the binding site for the MTHFR cofactor, flavin adenine dinucleotide (FAD) (7). Individuals with the *MTHFR C677T TT* genotype have been shown to have 30 percent in vitro MTHFR enzyme activity compared with the wild type, whereas those with the heterozygous (*CT*) genotype have been found to have 60 percent wild-type MTHFR enzyme activity (8). The *677T* allele has been associated with elevated plasma homocysteine levels (9), a somewhat increased risk of cardiovascular disease (9–12), and an increased risk of birth defects, especially neural tube defects (13–16). For a full review of the *C677T* polymorphism and its population frequencies, the reader is referred to the HuGE Review on MTHFR and congenital anomalies (15).

A1298C polymorphism

A second *MTHFR* polymorphism, *A1298C* in exon 7, results in a glutamate-to-alanine substitution at codon 429 (5, 13). This polymorphism lies in the *S*-adenosylmethionine-regulatory domain of the enzyme (17–19). The

TABLE 1. *MTHFR A1298C* polymorphism frequencies in different populations*

Study area and ethnic group	Total no.	Genotype (no.)			C allele frequency	Article
		AA	AC	CC		
Africa						
South Africa, Black indigenous†	114	70	39	5	0.21	Gebhardt et al., 2001 (107)
Asia						
China†	360	242	113	5	0.17	Song et al., 2001 (108)
China†	166	111	50	5	0.18	Shen et al., 2001 (109)
Japan†	243	159	75	9	0.19	Matsuo et al., 2001 (110)
Europe						
Austria†	389	184	168	37	0.31	Fodinger et al., 2001 (111)
Crete†	125	57	55	13	0.32	Zetterberg et al., 2002 (112)
Germany	280	128	123	29	0.32	Geisel et al., 2001 (113)
Germany†	174	88	68	18	0.30	Stegmann et al., 1999 (20)
Germany, Caucasians†	981	433	443	105	0.33	Meisel et al., 2001 (114)
Netherlands†	403	179	186	38	0.33	van der Put et al., 1998 (13)
Netherlands†	120	45	64	11	0.36	Lachmeijer et al., 2001 (115)
Netherlands†	565	250	258	57	0.33	Lievers et al., 2001 (116)
Poland†	521	316	180	25	0.22	Szczeklik et al., 2001 (117)
Poland†	100	55	41	4	0.24	Domagala et al., 2002 (118)
United Kingdom†	114	49	54	11	0.33	Skibola et al., 1999 (88)
United Kingdom†	200	93	83	23	0.32	Wiemels et al., 2001 (19)
United Kingdom	394	211	151	32	0.27	Dekou et al., 2001 (119)
Middle East						
Israel, Jewish	397	178	168	51	0.34	Friedman et al., 1999 (120)
North America						
United States						
Ashkenazi Jewish	149	80	57	12	0.27	Rady et al., 2002 (6)
Caucasians	159	70	75	14	0.32	Rady et al., 2002 (6)
Hawaii, Caucasians†	171	86	65	20	0.31	Rady et al., 2002 (121)
Hawaii, Japanese descent†	395	244	136	15	0.18	Rady et al., 2002 (121)
Midwest, unspecified ethnicities†	329	164	139	26	0.29	Hanson et al., 2001 (122)
Texas, Caucasians†	554	265	249	40	0.30	Shen et al., 2001 (123)
Male physicians†	344	153	159	32	0.32	Chen et al., 2002 (21)
Canada†	129	69	49	11	0.28	Ray et al., 2001 (23)
Canada†	119	43	67	9	0.36	Isotalo et al., 2000 (124)

* Only studies with more than 100 participants are included in this table. For the *MTHFR C677T* polymorphism frequencies, the reader is referred to the earlier HuGE Review by Botto and Yang (15).

† Case-control study. Only polymorphism frequencies for the control group are included in this table.

binding of *S*-adenosylmethionine (SAM) results in conformational changes within the MTHFR enzyme that inhibit the enzyme's activity (17). Lymphocytes from individuals with the 1298 CC genotype have been found to have approximately 60 percent specific wild-type in vitro MTHFR activity (13), and individuals with both 677 CT and 1298 AC genotypes were found to have 50–60 percent wild-type MTHFR activity (5, 13).

Population frequencies for the *A1298C* polymorphism are summarized in table 1. The C allele frequency ranges from 0.17–0.19 among Asian populations to 0.27–0.36 in western

Europe. Little is known about *A1298C* allele frequencies among African or South American populations.

The *MTHFR C677T* and *A1298C* sites are 2.1 kb apart and have been found to be in strong linkage disequilibrium (20, 21). Several groups have shown that the two polymorphisms very rarely exist on the same allele (5, 13, 20, 21), and the combined *MTHFR 677 TT* and *1298 CC* genotypes are extremely uncommon in the general population. These findings suggest a founder effect in which each alteration evolved on a separate wild-type allele (20–22).

T1317C polymorphism

The polymorphism identified at bp 1317 is a thymine-to-cytosine substitution, but it does not alter the amino acid sequence (5). The allele frequency for the *1317C* variant was 0.05 among a group of 38 Canadian women and 0.39 among nine African-American women (5). Ray et al. (23) reported a *1317C* allele frequency of 0.03 among 129 Canadian women serving as controls in a case-control study of genetic risk factors for thrombophilia.

G1793A polymorphism

In a study of 507 individuals representing several ethnic backgrounds randomly selected from clinic populations in Texas and New York, Rady et al. (6) recently reported a guanine-to-adenine substitution at bp 1793, resulting in an arginine-to-glutamine substitution at codon 594. They found an A allele frequency of 0.01 among Ashkenazi Jews ($n = 155$), 0.03 among African Americans ($n = 97$), 0.07 among Caucasians ($n = 159$), and 0.06 among Hispanics ($n = 95$) (6). The functional relevance of this polymorphism has not yet been investigated.

DISEASE

Leukemias, as a group, are cancers arising from hematopoietic cell lines. Genetic translocations, inversions, or deletions in hematopoietic cells disrupt the normal function of the genes at these locations, altering normal blood cell development (24). As a result, dysfunctional or nondifferentiated leukemic cells accumulate in the bone marrow space and progressively replace normal hematopoietic cells. Signs and symptoms of leukemia include anemia, fatigue, bleeding, and infections (25, 26). Leukemias can be either acute or chronic, and they can arise from myeloid or lymphoid cell lines, or both, as in the case of myeloid/lymphoid or mixed-lineage leukemia (MLL). The four major forms of leukemia are acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML) (27).

Acute leukemias are rare, accounting for only 2 percent of all cancers in the United States (28). Approximately 9,000 individuals are diagnosed with AML and 4,000 individuals are diagnosed with ALL annually in the United States (29). ALL occurs more commonly among children and young adults, with a median age at diagnosis of 10 years, whereas the median age of onset for AML is 65 years (30). CLL is the most common form of leukemia in adults in western countries, affecting approximately 10,000 individuals per year in the United States (30). CML affects approximately 4,000 individuals per year in the United States, with a median age at onset between the ages of 45 and 55 years (31). Leukemia is the most common type of cancer among children, with ALL accounting for 75 percent of leukemia cases, AML accounting for 20 percent, and CML accounting for less than 5 percent (32).

Although researchers have made significant strides in improving outcomes for individuals diagnosed with leukemia (33), very little is known about how the disease

originates. Exposure to ionizing radiation (34–36), benzene (37, 38), pesticides (39), cigarette smoking (40–42), and extremely low-frequency electromagnetic fields (39, 43) have all been found to be associated with an increased risk of leukemia in adults.

A large body of epidemiologic research has evaluated the association between infection and the risk of pediatric leukemias, particularly ALL. Kinlen (44) hypothesized that increased rates of leukemias are seen following periods of population mixing, which can introduce new infectious agents into a community of individuals who were previously unexposed or who may have susceptibility to the infectious agent. This theory has been corroborated by several studies reporting an increased risk of acute leukemias following periods of population mixing (45–52). Similarly, Greaves (53, 54) hypothesized that the development of leukemia is related to inadequate development of the infant's immune response or to a lack of exposure to infections in early childhood, resulting in an abnormal immune response when these children are later exposed to common infections (the "delayed infection" hypothesis). Although research using day-care attendance, sibship size, and birth order as surrogates for early exposure to common infections has shown inconsistent results (55–58), studies directly assessing the incidence of common infections report an inverse association between some, but not all, common infections, for example, ear infections (55), and the risk of leukemia (59). Down's syndrome has also been associated with an increased risk of pediatric leukemia (60, 61), primarily the acute myelogenous form (62).

Although single chromosomal aberrations have been identified as one of the causes of cellular dysfunction for many of the specific leukemia subtypes, it is likely that multiple genes and environmental factors play a role in an individual's susceptibility to these chromosomal aberrations (63). Dietary bioflavonoids, some of which are known topoisomerase II inhibitors, have been shown to cause site-specific DNA cleavage in the *MLL* gene breakpoint cluster region on chromosome 11q23 (64, 65). Maternal bioflavonoid intake during pregnancy has been associated with an increased risk of infant leukemias (66). A study by Thompson et al. (67) among 83 children with ALL and 166 age- and sex-matched controls found an inverse association between maternal folate supplementation during pregnancy and the risk of pediatric ALL, but they did not evaluate *MTHFR* polymorphisms. Rodent studies of chemical- or radiation-induced leukemia have found that caloric restriction (68–70), curcumin (71), and the monoterpene geraniol (72) may be protective against the development of leukemia.

ASSOCIATIONS

Biologic mechanisms

Folate, as a carrier of single carbon fragments, is an essential nutrient for normal mammalian cell growth. These reactions include purine and pyrimidine synthesis, as well as the provision of methyl groups for DNA, RNA, and protein methylation (73–75). Folate deficiency has been shown to result in uracil misincorporation during DNA replication

(76, 77) with subsequent increased double-strand breaks during uracil excision repair (78). Thus, an increased risk of leukemia-inducing translocations associated with low-folate status is conceivable.

Low-folate status has been associated with an increased risk of several types of cancer, especially colorectal cancer (73, 74, 79–82). Yet, *MTHFR* 677 TT individuals with adequate folate levels have been found to have a decreased risk of colorectal cancer (83–85). Several authors (83, 84, 86) have suggested that the explanation for this paradox is that, in situations of decreased MTHFR activity, more of its substrate (5,10-methylene-THF) is available for purine and pyrimidine synthesis, resulting in more stable DNA synthesis and decreased genetic mutations. This hypothesis was later supported by Bagley and Selhub (87), who observed that erythrocytes from individuals with the wild-type *MTHFR* 677 CC genotype contained mostly 5-methyl-THF, whereas individuals with the variant *MTHFR* 677 TT genotype contained both formylated THF and 5-methyl-THF. These findings demonstrate that decreased MTHFR activity results in an alteration of the normal intracellular distribution of folate substrates in favor of the precursors for purine and pyrimidine synthesis.

MTHFR polymorphisms and leukemia

Three case-control studies to date have addressed whether variant forms of the *MTHFR* gene alter the risk of leukemia (table 2). In a study of adult acute leukemia cases ($n = 71$ ALL, 237 AML; age range, 16–70 years) and controls ($n = 356$) conducted in the United Kingdom, Skibola et al. (88) found that individuals with the *MTHFR* 677 TT, 1298 AC, and CC genotypes have a decreased risk of ALL. The *MTHFR* 677 TT genotype was associated with a 4.3-fold decrease of ALL (odds ratio (OR) = 0.23, 95 percent confidence interval (CI): 0.06, 0.81; $n =$ five cases, 14 controls) compared with the 677 CC genotype, whereas a 14-fold decrease in risk (not significant) of ALL was observed among individuals with the *MTHFR* 1298 CC genotype (OR = 0.07, 95 percent CI: 0.00, 1.77; $n =$ one case, 11 controls) compared with the 1298 AA genotype. No statistically significant differences were observed in *MTHFR* genotypes among cases with AML and controls, suggesting that *MTHFR* polymorphisms do not play a role in the risk of AML. Odds ratios range from 0.73 to 1.00, and all confidence intervals include 1.0 (table 2).

Wiemels et al. (19) investigated whether the *MTHFR* C677T or A1298C polymorphism altered the risk of pediatric AML, MLL, or hyperdiploid leukemia. The study population included 253 cases (78 cases of AML, 37 cases of MLL, and 138 cases of hyperdiploid leukemia) who were less than 15 years of age and participants in the United Kingdom Childhood Cancer Study and 200 unselected newborn controls from the Manchester, United Kingdom, area. They found that the *MTHFR* 677 CT and TT genotypes (but not the 1298 AC and CC genotypes) were associated with a significantly decreased risk of leukemias with MLL rearrangements compared with the wild type (OR = 0.36, 95 percent CI: 0.15, 0.85; $n =$ 11 cases, 111 controls). The variant *MTHFR* 1298 CC genotype (but not the 677 TT genotype) was asso-

ciated with a significantly decreased risk of hyperdiploid leukemias (leukemias in which the aberrant cells have accumulated additional chromosomes) (OR = 0.26, 95 percent CI: 0.07, 0.81; $n =$ five cases, 23 controls). Neither of the two major *MTHFR* polymorphisms appeared to be associated with AML (table 2). A smaller study (71 cases, 71 controls; age range, from 2 months to 15 years) of pediatric ALL from Brazil found that the presence of the *MTHFR* 677 T allele was associated with a 2.4-fold decreased risk of ALL (OR = 0.4, 95 percent CI: 0.2, 0.8; $n =$ 34 cases, 49 controls), whereas the A1298C genotype did not significantly affect the risk of ALL (89).

These studies suggest that the altered distribution of intracellular folate metabolites introduced by the variant forms of *MTHFR* may play a role in the risk of lymphoid forms of leukemia but not myeloid leukemias. However, the sample sizes for disease subtypes in these studies were small, and the findings need to be confirmed in larger populations. Although it is not entirely clear why this disparity between the lymphoid and the myeloid forms of the disease has been observed, it suggests that differences in folate requirements or susceptibility to chromosomal damage may exist between myeloid and lymphoid cells. One group has suggested that lymphoid cells may have a higher folate requirement than do myeloid cells and, therefore, may be more susceptible to DNA damage as a result of folate deficiency than are myeloid cells (88). None of the studies to date has assessed dietary folate intake to evaluate whether the overall folate status may have modified the relation between the *MTHFR* genotype and the risk of leukemia.

INTERACTIONS

Gene-environment interactions

Conflicting results among studies investigating *MTHFR* polymorphisms and the risk of various forms of cancer may be due to the lack of information on dietary folate intake or other measures of overall folate status. With respect to colorectal carcinogenesis, studies that have investigated both *MTHFR* polymorphisms and dietary folate intake or plasma folate levels have found a trend toward homozygous variants of *MTHFR* genotypes' adversely affecting adenoma or cancer risk only in individuals with low dietary folate intake or plasma folate levels (84, 86, 90). Individuals with the *MTHFR* 677 TT genotype with normal plasma folate concentrations have been found to be at decreased risk of colorectal cancer relative to those with the CC genotype (83, 84). These findings demonstrate that the risk associated with the *MTHFR* 677 TT genotype varies depending on folate status. There have been no reports to date describing the effects of dietary folate intake on the association between the *MTHFR* 1298 polymorphism and the risk of cancer.

Dietary intake of several other nutrients could also affect the distribution of intracellular folate metabolites. Vitamins B₆ and B₁₂ are cofactors for the enzymes serine hydroxymethyltransferase (SHMT) and methionine synthase (MS), respectively. Dietary adequacy of these nutrients may affect substrate availability for DNA synthesis and MTHFR activity. Dietary intake of protein-rich foods (especially

TABLE 2. Summary of studies investigating *MTHFR* polymorphisms and leukemia risk

Article	Study population	Findings
Skibola et al., 1999 (88)	308 acute adult leukemia cases (71 ALL, 237 AML*), 491 controls Cases were 16–70 years of age Controls randomly selected from patients under the care of the same local physician as the case and matched by sex, year of birth (± 2 years), and race	ALL* \dagger <i>MTHFR 677</i> CT vs. CC (OR* = 0.58, 95% CI*: 0.27, 1.28; $n = 29$ cases, $n = 39$ controls) TT vs. CC (OR = 0.23, 95% CI: 0.06, 0.81; $n = 5$ cases, $n = 14$ controls) <i>MTHFR 1298</i> AC vs. AA (OR = 0.33, 95% CI: 0.15, 0.73; $n = 23$ cases, $n = 54$ controls) CC vs. AA (OR = 0.07, 95% CI: 0.00, 1.77; $n = 1$ case, $n = 11$ controls) Combined <i>MTHFR 677/1298</i> No significant interactions; at lowest risk were individuals with 677 CC/1298 CC (OR = 0.07, 95% CI: 0.00, 1.77; $n = 1$ case, $n = 11$ controls) and 677 CT/1298 AC (OR = 0.18, 95% CI: 0.04, 0.75; $n = 5$ cases, $n = 17$ controls) genotypes AML \dagger <i>MTHFR 677</i> CT vs. CC (OR = 0.73, 95% CI: 0.48, 1.11; $n = 66$ cases, $n = 130$ controls) TT vs. CC (OR = 0.84, 95% CI: 0.43, 1.62; $n = 23$ cases, $n = 43$ controls) <i>MTHFR 1298</i> AC vs. AA (OR = 0.99, 95% CI: 0.66, 1.50; $n = 98$ cases, $n = 157$ controls) CC vs. AA (OR = 0.95, 95% CI: 0.51, 1.76; $n = 23$ cases, $n = 40$ controls)
Wiemels et al., 2001 (19)	253 cases of pediatric leukemia (138 hyperdiploid leukemia, 78 AML, 37 MLL), 200 newborn controls Cases ≤ 15 years of age Controls consisted of umbilical cord blood samples from unselected, healthy, newborn infants in the Manchester, United Kingdom, area	MLL* \dagger <i>MTHFR 677</i> CT vs. CC (OR = 0.29, 95% CI: 0.09, 0.79; $n = 6$ cases, $n = 79$ controls) TT vs. CC (OR = 0.67, 95% CI: 0.16, 2.53; $n = 5$ cases, $n = 32$ controls) CT + TT vs. CC (OR = 0.36, 95% CI: 0.15, 0.85; $n = 11$ cases, $n = 111$ controls) <i>MTHFR 1298</i> AC vs. AA (OR = 1.35, 95% CI: 0.53, 3.63; $n = 18$ cases, $n = 83$ controls) CC vs. AA (OR = 1.33, 95% CI: 0.32, 5.29; $n = 6$ cases, $n = 23$ controls) AC + CC vs. CC (OR = 1.14, 95% CI: 0.49, 2.73; $n = 24$ cases, $n = 106$ controls) Hyperdiploid leukemia \dagger <i>MTHFR 677</i> CT vs. CC (OR = 0.82, 95% CI: 0.49, 1.38; $n = 6$ cases, $n = 79$ controls) TT vs. CC (OR = 0.49, 95% CI: 0.20, 1.17; $n = 5$ cases, $n = 32$ controls) CT + TT vs. CC (OR = 0.82, 95% CI: 0.51, 1.34; $n = 71$ cases, $n = 111$ controls) <i>MTHFR 1298</i> AC vs. AA (OR = 0.98, 95% CI: 0.58, 1.64; $n = 67$ cases, $n = 83$ controls) CC vs. AA (OR = 0.26, 95% CI: 0.07, 0.81; $n = 5$ cases, $n = 23$ controls) AC + CC vs. CC (OR = 0.92, 95% CI: 0.57, 1.49; $n = 72$ cases, $n = 106$ controls) AML \dagger <i>MTHFR 677</i> CT vs. CC (OR = 1.09, 95% CI: 0.56, 2.12; $n = 34$ cases, $n = 79$ controls) TT vs. CC (OR = 0.91, 95% CI: 0.33, 2.44; $n = 13$ cases, $n = 32$ controls) CT + TT vs. CC (OR = 1.03, 95% CI: 0.551, 1.92; $n = 47$ cases, $n = 111$ controls) <i>MTHFR 1298</i> AC vs. AA (OR = 0.58, 95% CI: 0.30, 1.13; $n = 23$ cases, $n = 83$ controls) CC vs. AA (OR = 0.56, 95% CI: 0.16, 1.76; $n = 6$ cases, $n = 23$ controls) CT + TT vs. CC (OR = 0.59, 95% CI: 0.31, 1.08; $n = 29$ cases, $n = 106$ controls)
Franco et al., 2001 (89)	71 pediatric ALL cases, 71 controls Cases ≤ 15 years of age Controls matched by age, gender, and race	<i>MTHFR 677</i> CT vs. CC (OR = 0.5, 95% CI: 0.2, 0.9; $n = 28$ cases, $n = 36$ controls) TT vs. CC (OR = 0.3, 95% CI: 0.09, 0.8; $n = 6$ cases, $n = 13$ controls) CT + TT vs. CC (OR = 0.4, 95% CI: 0.2, 0.8; $n = 34$ cases, $n = 49$ controls) <i>MTHFR 1298</i> AC vs. AA (OR = 1.3, 95% CI: 0.7, 7.6; $n = 36$ cases, $n = 22$ controls) CC vs. AA (OR = 2.8, 95% CI: 0.5, 15.6; $n = 5$ cases, $n = 2$ controls) AC + CC vs. AA (OR = 1.3, 95% CI: 0.7, 2.6; $n = 35$ cases, $n = 30$ controls) <i>MTHFR 677 CT/1298 AC</i> (OR = 0.8, 95% CI: 0.4, 1.9; $n = 13$ cases, $n = 15$ controls)

* ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; OR, odds ratio; CI, confidence interval; MLL, myeloid/lymphoid or mixed lineage leukemia.

\dagger Multivariate analysis controlling for the respective other *MTHFR* polymorphism.

meats, fish, and cheeses) will influence the supply of the amino acid methionine. Chronic, excessive use of alcohol may take the place of more nutrient-dense foods in the diet, leading to deficiencies in folate and other B vitamins. Alcohol intake may also affect folate absorption, metabolism, and renal excretion (91). Although these gene-nutrient interactions have not been studied with respect to the risk of leukemia, studies have found that individuals with the

MTHFR 677 TT genotype and diets low in vitamins B₆ and B₁₂, methionine, and folate and high in alcohol are at increased risk of developing colorectal tumors (83, 85, 86, 90, 92).

The amino acid affected by the *MTHFR C677T* polymorphism lies at the base of the binding site for flavin adenine dinucleotide (FAD), a cofactor for the *MTHFR* enzyme (7). The thermolabile form of the *MTHFR* enzyme (677 *TT*

genotype) has been shown to dissociate with the FAD cofactor more readily than the wild-type enzyme, resulting in decreased enzyme activity (7). In situations where there is abundant intracellular folate, the folate molecule may be able to hold the variant MTHFR protein in the appropriate and functional three-dimensional structure (7, 93).

As FAD is a form of the B vitamin riboflavin, riboflavin status has also been suggested as playing a role in optimal MTHFR activity. Two studies to date have investigated the relation between riboflavin and *MTHFR* genotypes using total plasma homocysteine levels as the outcome variable of interest. Hustad et al. (94) found plasma riboflavin levels to be an independent determinant of plasma homocysteine levels in a study of 423 healthy blood donors between the ages of 19 and 69 years in Norway. However, in a subsequent study, Jacques et al. (95) reported that plasma riboflavin status affected only total plasma homocysteine levels among individuals with both the *MTHFR* 677 TT genotype and low plasma folate levels in a subset of 450 participants in the Framingham Offspring Study cohort. Neither study evaluated the effects of dietary riboflavin and folate on MTHFR activity or total plasma homocysteine levels.

Gene-gene interactions

Other proteins involved in folate-mediated, one-carbon metabolism, such as MS, thymidylate synthase (TS), SHMT, dihydrofolate reductase, and the reduced folate carrier protein, have been well characterized. Each plays a role in the regulation of the various forms of intracellular folate, and variant forms of these enzymes may contribute to the risk of DNA instability and cancer. As shown in figure 1, folate metabolism is complex and involves several regulatory mechanisms. Thus, genetic variation affecting protein function at any step may alter the balance of metabolites, and gene-gene interactions between polymorphisms in folate-metabolizing enzymes are likely. A detailed review of folate metabolism and its genetic variability is beyond the scope of this review, and the reader is referred elsewhere (96).

In a further analysis of their case-control study of adult patients with ALL, Skibola et al. (63) investigated the association between polymorphisms in the *MS*, cytosolic *SHMT* (*cSHMT*), and *TS* genes and the risk of adult ALL. Univariate analysis showed that individuals who were either homozygous or heterozygous for the variant forms of the *cSHMT* or *TS* gene had a decreased risk of ALL; however, no significant associations were observed for the *MS* polymorphism. When interactions between the *cSHMT* polymorphism and the *TS* or *MS* gene were evaluated, the variant *cSHMT* allele was associated with a greater than 13-fold decreased risk of ALL among individuals with a homozygous triple repeat in the promoter region of the *TS* gene (OR = 0.07, 95 percent CI: 0.0067, 0.77) and with a 5.6-fold decreased risk of ALL among individuals who were heterozygous for the variant form of the *MS* gene (OR = 0.18, 95 percent CI: 0.05, 0.63). Although these investigations of gene-gene interaction were hampered by small sample sizes, they illustrate the potential for interaction between multiple genetic variants in folate metabolism.

LABORATORY TESTS

MTHFR genotype determinations can be assessed by direct sequencing or restriction fragment length polymorphism assays as discussed in the previous HuGE Review on *MTHFR* gene variants and congenital abnormalities (15). All of the studies reviewed in this article reported using polymerase chain reaction/restriction fragment length polymorphism analysis for genotyping. More recently, multiplexed genotyping for simultaneous assessment of multiple polymorphism sites including *MTFHR* has been reported (97–99), although no data on the specificity, sensitivity, and predictive value of these testing methods are available.

POPULATION TESTING

There is still too little known about the disease risk associated with genetic polymorphisms in folate-metabolizing enzymes to advocate population testing at the current time. As previously mentioned, adequate dietary folate intake may be sufficient to overcome any potential detrimental effects of variant *MTHFR* transcripts, and public health measures to encourage increased dietary folate consumption appear to be the most beneficial approach at this time. Larger studies of leukemia that take folate status into account are needed.

GAPS AND RESEARCH PRIORITIES

Research into the role of *MTHFR* polymorphisms in hematologic malignancies is still in the early stages. We suggest these additional research priorities.

Larger sample sizes are needed. Leukemias are relatively rare malignancies, accounting for only 2 percent of all new cancer cases predicted to be reported in the United States in 2002 (28). As a result, studies to date have been relatively small. Small studies often lack adequate representation in certain genotype groups, cannot adequately address gene-gene or gene-environment interactions, and can also be subject to publication bias. Large, well-designed studies are needed and will likely require multicenter or multinational collaborations.

Gene-environment interactions must be addressed. There is sufficient evidence from studies investigating the role of *MTHFR* polymorphisms in other diseases indicating that intracellular folate levels may modify the effects of the *MTHFR* C677T and A1298C polymorphisms. Studies that do not assess folate status (e.g., by measuring dietary intakes or using biomarkers) may not be able to discern the true associations between *MTHFR* polymorphisms and leukemia risk. An assessment of folate status should be included in studies investigating genetic polymorphisms in folate-metabolizing enzymes.

Consider genetic variability in several proteins in folate metabolism concurrently. Folate metabolism involves several key enzymes that all contribute to the intracellular folate flux. It is likely that, if folate status truly influences the risk of leukemia, a better understanding of genetic variability within multiple proteins in the pathway will help to more precisely assess an individual's risk level.

Further exploration into the structure and properties of the various polymorphic forms of human *MTHFR* is needed. No one has been able to express sufficient quantities of the human *MTHFR* protein to allow for structural determinations to date. Therefore, our understanding of *MTHFR* structure and function has been extrapolated from other organisms, such as *Escherichia coli* (7), an organism with 34 percent sequence homology with the human (Unigene cluster Hs. 214142). Structure information may provide a molecular basis for the observation of decreased activity of the variant forms of *MTHFR*, demonstrate the degree to which each polymorphism affects the function of the molecule, and elucidate the role that environmental factors such as intracellular levels of folate and riboflavin play in regulating *MTHFR* activity.

Investigate gene-environment interactions in utero. Interactions among maternal and fetal *MTHFR* polymorphisms, maternal folate intake during pregnancy, and their effects on the subsequent risk of pediatric leukemia also warrant further exploration. It is known that the initiation of infant leukemias can occur in utero from the findings of leukemic translocations in neonatal blood spots (100–102), the presence of leukemia in newborns, and the occurrence of monozygotic twins with identical leukemic rearrangements (103–106). However, a recent report by Mori et al. (102) found that the presence of the common leukemia fusion genes *TEL-AML1* and *AML1-ETO* among healthy newborns is 100-fold greater than the risk of leukemia, indicating that additional genetic and/or environmental exposures are required for the development of leukemia.

CONCLUDING REMARKS

Although studies investigating the association between *MTHFR* genetic polymorphisms and the risk of leukemia to date have lacked statistical power, their results are intriguing and quite consistent. Future studies should have adequate statistical power for investigating combinations of the two *MTHFR* polymorphisms and those of other folate-metabolizing enzymes, as well as alterations in risk depending on folate status.

INTERNET SITES

Internet sites pertaining to leukemia and genetic mutations are listed in appendix table 1.

ACKNOWLEDGMENTS

Support for K. R. was provided by training grant T32 CA80416 from the National Cancer Institute.

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(Appendix follows.)

APPENDIX

APPENDIX TABLE 1. Internet sites pertaining to leukemia and genetic mutations

Type of site	Sponsor	Title	World Wide Web URL
Online Mendelian Inheritance in Man	National Center for Biotechnology Information	5,10-Methylenetetrahydrofolate reductase; MTHFR	http://www.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=236250
ENZYME	Swiss Institute of Bioinformatics	NiceZyme View of ENZYME: EC 1.5.1.20 (methylenetetrahydrofolate reductase)	http://www.expasy.ch/cgi-bin/nicezyme.pl?1.5.1.20
SWISS-PROT	Swiss Institute of Bioinformatics	NiceProt View of SWISS-PROT: P42898 (MTHR_Human)	http://www.expasy.ch/cgi-bin/niceprot.pl?P42898
SNP500Cancer Database	National Cancer Institute		http://snp500cancer.nci.nih.gov/home.cfm
Cancer Genome Anatomy Project	National Cancer Institute		http://cgap.nci.nih.gov/
Environmental Genome Project	National Institute of Environmental Health Sciences		http://www.niehs.nih.gov/envgenom/home.htm
Leukemia Home Page	National Cancer Institute		http://www.nci.nih.gov/cancer_information/cancer_type/leukemia/
The Leukemia and Lymphoma Society			http://www.leukemia.org/hm_lls