To the editor:

MTHFR genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia

In a recent report on the role of the *5,10-methylenetetrahydrofolate reductase (MTHFR)* genetic polymorphisms in childhood acute lymphoblastic leukemia (ALL), Krajinovic et al reported a reduced risk associated with a combination of genotypes.[1] On stratification of patients into those born before and after January 1996 (when the effects of recommended folate supplementation during pregnancy could be observed), Krajinovic et al observed that the protective effect was present only in children born before 1996.[1] These results suggested that the associated risk with a combination of genotypes in the *MTHFR* gene was dependent on dietary folate status. Similarly, a protective effect of variant genotypes of these polymorphisms was reported in various subtypes of childhood acute leukemia in an earlier study.[2] *MTHFR* gene polymorphisms also were previously shown to modulate the risk of adult acute leukemia.[3] In a recent study, the C677T variant was associated with a relapse of childhood ALL.[4]

Childhood leukemias are rare malignancies, and studies to-date on the effect of *MTHFR* genetic polymorphisms have been relatively small and a case for larger studies has been argued.[5] In the largest study thus far on childhood acute leukemia,[6] we investigated distribution of *MTHFR* genetic variants C677T and A1298C in 460 German patients and 1472 controls matched for ethnicity. The prevalent cases recruited were born between 1983 and 2003, with a mean diagnosis age of 6.9 years (± 4.4 years). The polymorphisms were analyzed with TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA), and results were validated by random DNA sequencing.

Our results showed that frequencies for the variant alleles T677 and C1298 were in concordance with those reported for the European population and genotypes followed the Hardy-Weinberg distribution.[7] Contrary to Krajinovic et al,[1] we found no statistically significant difference in genotype frequencies for

Table 1. Distribution of MTHFR genotypes in childhood ALL patients and controls

Genotype	ALL cases, no. (%)	Controls, no. (%)	OR* (95% CI)	*P*
MTHFR C677T genotype†				
CC	199 (43.9)	600 (41.4)	1.00 (referent)	–
CT	195 (43.0)	681 (47.0)	0.86 (0.7–1.1)	.2
TT	59 (13.0)	167 (11.5)	1.07 (0.8–1.6)	.8
CT + TT	254 (56.1)	848 (58.6)	0.90 (0.7–1.1)	.4
C-allele	593 (65.5)	1881 (65.0)	1.00 (referent)	–
T-allele	313 (34.5)	1015 (35.0)	0.98 (0.8–1.2)	.8
MTHFR A1298C genotype‡				
AA	198 (44.5)	660 (45.4)	1.00 (referent)	–
AC	195 (43.0)	644 (44.3)	1.01 (0.8–1.3)	1
CC	52 (11.7)	149 (10.2)	1.16 (0.8–1.7)	.5
AC + CC	247 (55.5)	793 (54.5)	1.04 (0.8–1.3)	.8
A-allele	591 (66.4)	1964 (67.6)	1.00 (referent)	–
C-allele	299 (33.6)	942 (32.4)	1.05 (0.9–1.2)	.5
Genotype combination				
C677T/A1298C§				
CC/AA	45 (10.2)	150 (10.5)	1.00 (referent)‖	–
CC/AC	98 (22.3)	296 (20.6)	1.10 (0.7–1.7)	.7
CC/CC	51 (11.6)	149 (10.4)	1.14 (0.7–1.9)	.7
CT/AA	93 (21.2)	339 (23.6)	0.91 (0.6–1.4))	.7
CT/AC	95 (21.6)	335 (23.3)	0.95 (0.6–1.4)	.6
CT/CC	1 (0.02)	-	NE	–
TT/AA	57 (13.0)	166 (11.6)	1.14 (0.7–1.8)	.5

– indicates none; NE, not estimated.

*Allelic and genotype frequencies for both polymorphisms and frequencies of genotype combinations in cases and controls were compared by Yates corrected χ^2-test. Odds ratios (ORs) for genotype frequencies between cases and controls and 95% confidence intervals (95% CI) were calculated by logistic regression using SAS version 8.2 software (SAS Institute, Heidelberg, Germany).

†ALL (n = 453); controls (n = 1448). The number of cases recruited in the study was 460; however, genotype results could not be determined in 7 cases for C677T and in 15 cases for A1298C polymorphisms. Similarly, of 1472 controls, genotype for C677T and A1298C polymorphisms could not be determined in 24 and 19 individuals, respectively.

‡ALL (n = 445); controls (n = 1453).

§ALL (n = 440); controls (n = 1435).

‖The risk of CC677/AA1298 individuals compared with all other genotypes is 0.98 (95% CI, 0.7–1.4; *P* = .96); no individual with TT677/AC1298 and TT677/CC1298 genotype combinations was observed.

the A1298C polymorphism between cases and controls. The frequency of the CC677/CC1298 genotype combination in cases was 11.6% and in controls 10.4% (Table 1). Similarly, we found no statistically significant difference in frequency of TT677/AA1298 genotype combination between cases (13%) and controls (11.6%). Both of these genotype combinations were shown to have a protective effect in the earlier study on acute leukemia.[1] A review of various dietary recommendations suggested emphasis on supplementary folate during pregnancies in Germany from the mid-1990s, similar to that reported for Canada by Krajinovic et al.[1] On this assumption, we stratified our cases into those born before 1996 and those born in 1996 and onward. However, we did not find any statistically significant differences in either genotype or combination of genotype frequencies among cases in 2 strata and controls (data not shown). The sample size in our study was large enough to detect an odds ratio (OR) of 0.5 or less with more than 90% power.

Multiple factors could be the reason for differences between our observations in this study and the results reported by Krajinovic et al[1] and others.[2,3] Those include differences in population and dietary folate and other nutrient intake. We conclude from our observation that polymorphisms in the *MTHFR* gene, per se, are not likely to modulate susceptibility to childhood ALL in Germany.

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Response:

MTHFR genetic variants: an example of gene-nutrient interaction and susceptibility to childhood acute lymphoblastic leukemia

In the letter by Kumar and colleagues in this issue of *Blood*, the authors failed to reveal any association between *MTHFR* genetic variants (677C>T and 1298A>C) and the risk of childhood acute lymphoblastic leukemia (ALL) in an association study targeting 460 patients recruited between 1983 to 2003 and 1472 controls of German origin. As mentioned by the authors, factors related to both genetics and nutrition might explain the discrepancies between Kumar et al's data and those reported by other groups.[1-4]

MTHFR is one of the best examples of a polymorphic gene investigated in the context of cancer susceptibility, particularly leukemia (see Robien and Ulrich[5] for further discussion). This might be explained by the availability of several groups of patients worldwide that have been used to validate genetic epidemiology data.

In a French-Canadian cohort (n = 271 patients), we observed an underrepresentation of CC1298 homozygotes among the ALL patients compared with controls (4.4% vs 10.3%, respectively), suggesting a protective effect of this variant (odds ratio [OR] = 0.4;95% CI, 0.2-0.8; $P < .01$).[6] The analysis of both genotypes showed that CC677/AA1298 individuals were at a higher risk of ALL compared with those with other genotypes (OR = 1.8; 95% CI, 1.1-2.8; $P = .02$). This led to the suggestion that carriers of alleles T677 and C1298 have a decreased susceptibility to ALL, particularly the homozygotes for either one or another of these variants: TT677/AA1298 (OR = 0.4; 95% CI, 0.2-0.9; $P = .02$) or CC677/CC1298 (OR = 0.3; 95% CI, 0.1-0.6; $P < .001$). Similar results were obtained with a parental trio design, thus decreasing putative population stratification bias. These data confirm and extend previous findings by Skibola et al,[3] who reported reduced frequency of the same *MTHFR* haplotypes in adult leukemia (n = 71 patients from United Kingdom), as well as results of Wiemels et al,[2] who found the protective effect of these variants in infant leukemia with *MLL* rearrangements and hyperdiploid pediatric leukemia (n = 253 patients from United Kingdom). Furthermore, Franco et al[4] reported in a group of 71 Brazilian patients a protective effect of the T677 variant. Taken together, these 4 studies including 666 ALL patients from 4 distinct cohorts observed similar protective effects of at least one of the tested MTHFR variants, suggesting that population-to-population differences are a less plausible explanation; although, an apparent European north-to-south range of frequencies has been reported.[7]

Considering the reported protective association between folate supplements in pregnancy and the risk of common childhood ALL,[8] maternal diet might represent a suitable determinant of the disease, particularly for the effects of *MTHFR* variants that seem to be modified by folate levels. Indeed, we observed a protective effect in TT677/AA1298, CC677/CC1298, and CC677/AC1298 only in children born before Health Canada's recommendation to give folate supplements during pregnancy, thus presumably reflecting the maternal folate insufficiency during pregnancy.[6] However, Kumar et al did not observe such association. In this regard, it is worth mentioning that a study addressing the prenatal vitamin supplementation issue reported substantial differences in the number of mothers taking vitamins during pregnancy, ranging from 3% in France to 90% in US centers.[9] We propose that such gene-nutrient interaction might explain, at least in part, the differences observed between genetic association studies.

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To the editor:

Final height after transplantation in childhood

With interest we read the paper by Sanders et al[1] in which it is concluded that growth hormone (GH) therapy can increase final height in young children after hematopoietic cell transplantation (HCT). We do not disagree with the general conclusion that GH is efficacious in these children, but we would like to make several comments.

The paper does not offer information on the GH assay used. GH immunoassays show poor interassay agreement, with interassay variability up to 330%, depending on assays and international standard reference preparations (66/217 1 mg = 2 IU; 80/505 1 mg = 2.6 IU; 88/624 1 mg = 3.0 IU) used.[2] With most modern assays and new reference standards, the historical cutoff levels for peak GH after pharmacologic stimulation (7-10 ng/mL) should be reduced by 30% to 50%.[2,3] Without information on assay and pulse-detection method used, interpretation of the 3 criteria for normal spontaneous (assumed nocturnal) GH secretion is impossible. Both the high incidence (84%) of GH deficiency (GHD) in patients routinely tested and references for spontaneous GH secretion from the literature justify concerns about the diagnostic criteria. With regard to the criterion of more than 3 spontaneous peaks, we would like to point out that in non–GH-deficient children the mean number of peaks is 4 to 5, with 3 peaks in a substantial number of children.[4-8] In addition, in GHD (radiation induced or idiopathic) the peak amplitude is decreased, but peak frequency is preserved.[7-9] With respect to the criterion that the highest peak should be more than 18 ng/mL (we assume that ng/dL is an administrative error), we comment that many studies report even lower mean or median values for maximum peak GH in non–GH-deficient children.[6-8,10,11] As there is no evidence that the spontaneous nocturnal peak GH is higher than the peak GH after pharmacologic stimulation, we believe that the cutoff for maximum GH should be the same for spontaneous and stimulated GH secretion (8.6 ng/mL). With respect to the criterion that the mean GH should be more than 3 ng/mL, we acknowledge that this cutoff was used in several older studies, but in more recent studies[4,6-8,10,11] it is comparable with the mean (or median) value for average nocturnal GH levels in non–GH-deficient children (median < mean due to skewed distribution of results).

Apart from the definition of GHD, we question the definition of final height (height measured closest to age 16 years). In healthy boys, there is a more than 3-cm difference between height at age 16 years and final height, and height velocity at age 16 years is approximately 3 cm/y. Therefore the article of Sanders et al[1] reports about height at age 16 years rather than about final height. As this definition is used in both treated and untreated groups of patients, the results of the group comparison are still valid, but then only for the height at age 16 years. Actual final height could differ by 0.5 SD in boys.

GH treatment had a significant effect in children undergoing HCT before the age of 10 years, but not at more advanced ages. One could argue that age at start of GH therapy is more likely to influence the effect of GH therapy on final height than age at HCT: starting GH therapy at age 15.9 years (as one patient did) will probably have less effect than starting GH at age 12 years, irrespective of age at HCT. We are very interested in the effect of age at diagnosis of GHD/start of GH on final height, which could be helpful in deciding which patients should be considered for GH therapy.

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