5,10-Methylenetetrahydrofolate Reductase Polymorphisms and Acute Lymphoblastic Leukemia Risk: A Meta-analysis

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Abstract

There is evidence supporting a role for 5-10 methylenetetrahydrofolate reductase (*MTHFR*) gene variants in acute lymphoblastic leukemia (ALL). To provide a more robust estimate of the effect of *MTHFR* polymorphisms on the risk of ALL, we did a meta-analysis to reevaluate the association between the two most commonly studied *MTHFR* polymorphisms (C677T and A1298C) and ALL risk. All case-control studies investigating an association between the C677T or A1298C polymorphisms and risk of ALL were included. We applied both fixed-effects and random-effects models to combine odds ratio (OR) and 95% confidence intervals (95% CI). Q-statistic was used to evaluate the homogeneity and both Egger and Begg-Mazumdar tests were used to assess publication bias. The meta-analysis of the C677T polymorphism and risk of childhood ALL included 13 studies with a total of 4,894 individuals. Under a fixed-effects model, the TT genotype failed to be associated with a statistically significant reduction of childhood ALL risk (TT versus CT + CC: OR, 0.88; 95% CI, 0.73-1.06; $P = 0.18$). However, individuals homozygous for the 677T allele exhibited a 2.2-fold decrease in risk of adult ALL (TT versus CT + CC: OR, 0.45; 95% CI, 0.26-0.77; $P = 0.004$). In both cases, no evidence of heterogeneity was observed. No association between the A1298C variant and susceptibility to both adult and childhood ALL was disclosed. Our findings support the proposal that the common genetic C677T polymorphism in the *MTHFR* contributes to the risk of adult ALL, but not to the childhood ALL susceptibility. (Cancer Epidemiol Biomarkers Prev 2006;15(10):1956–63)

Introduction

Acute lymphoblastic leukemia (ALL) is the commonest pediatric cancer in industrialized countries (1, 2). With an incidence expected to reach up to 4.75 cases per 100.000 people worldwide, ALL represents ~80% of leukemia diagnoses (2). Whereas ALL accounts for 23% of cancers among children younger than 15 years, it is responsible for up to 20% of all adult leukemias, which are characterized by a worse prognosis with a decreased long-term survival (2, 3). Although a significant improvement in both ALL diagnosis and treatment has been made over the past decades, the etiology of most cases of ALL remains unknown due to probable multifactorial mechanisms of pathogenesis (4). Recently, however, molecular epidemiologic case-control studies suggest that both adults and children harboring variant alleles of the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene might have a decreased risk of ALL development (5).

The gene coding for MTHFR enzyme is located at chromosome 1.p36.3 and composed of 2.2 kilobases with a total of 11 exons (6). Despite the fact that several *MTHFR* polymorphisms have been described thus far, only two polymorphisms, C677T and A1298C, have been intensively investigated. The C-to-T transition at the nucleotide position 677 in exon 4 of *MTHFR* generates an alanine-to-valine substitution at amino acid 222. This substitution lies at the binding site for the flavin adenine dinucleotide (7), an important cofactor for MTHFR. As a result, carriers of the *MTHFR* 677TT genotype possess a thermolabile enzyme of reduced activity (8), with a subsequent mild decrease in both serum and plasma folate and an increase in homocysteine levels (5, 9). The second most studied polymorphism in *MTHFR* is an A-to-C transversion substitution at nucleotide 1,298 (exon 7) that results in an amino acid substitution of glutamate for alanine at codon 429 (10). Once this amino acid substitution takes place at the *S*-adenosylmethionine regulatory domain of the MTHFR, the A1298C polymorphism also generates an enzyme with a decreased activity (10). However, in contrast to the C677T variant, biochemical observations indicate that individuals homozygous for the 1298C allele do not seem to have higher serum homocysteine levels or any modification in folate status when compared with the wild-type genotype (9).

The biological mechanism by which *MTHFR* variants are thought to be associated with the ALL risk modulation is related to a more efficient DNA synthesis (5). Because folate plays a major role in normal human cell growth and leukemias commonly arise as a result of DNA translocations, inversions, or deletions in regulatory genes of the blood cell development and homeostasis (4, 5, 11), a decreased MTHFR activity might result in an alteration of the normal intracellular distribution of folate substrates (12). As a result, the folate precursors pathway would be directed for purine and pyrimidine synthesis, generating a reduced uracil misincorporation (13) with a subsequent decreased number of genetic mutations and a more stable DNA synthesis. Despite the appealing biological mechanism, results on the relation between *MTHFR* polymorphisms and ALL risk are derived mainly from underpowered studies and yielded conflicting results. To investigate the possible effect of *MTHFR* variants on the ALL risk through a more robust and powered analysis, we did a meta-analysis of 13 molecular epidemiologic case-control studies that examined

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the association between *MTHFR* polymorphisms and risk of both adult and childhood ALL.

Materials and Methods

Literature Search. Published reports examining the effect of the C677T and/or A1298C MTHFR polymorphisms on the risk of both adult and childhood ALL were identified through electronic searches in MEDLINE, Biological Abstracts, EMBASE, Web of Science, and Lilacs databases. Databases searches were done from January 1999 (the year that *MTHFR* polymorphisms were first associated with risk modulation in ALL) to April 2006. Electronic database searches were supplemented by manual searches of references of published papers and review articles. The abstracts from major hematologic disease meetings within the past 5 years were screened and experts in the field were contacted to search for unpublished studies. Studies were retrieved through an intensive combination of both MeSH terms and part of the text words and titles. The following terms were used: ''Methylenetetrahydrofolate Reductase,'' ''genotype,'' ''Leuk(a)emia,'' ''Acute lymphocytic,'' ''Acute lymphoblastic,'' ''Childhood,'' ''P(a)ediatric,'' ''polymorphism,'' ''MTHFR,'' ''C677T,'' ''A1298C,'' ''folate,'' and ''mutation.'' Literature search included all languages.

Selection Criteria. For inclusion, studies had to be case-control in design, involve unrelated subjects, and examine the association between ALL and the presence of the C677T or A1298C *MTHFR* variants. Exclusion criteria were as follows: (*a*) study design other than case-control (e.g., single case reports, cohort study design without control group); (*b*) main outcome other than the risk of ALL among genotypes (e.g., pharmacogenetic studies); and (*c*) control group not in accordance with Hardy-Weinberg equilibrium (14). Reports were further excluded if they evaluated the role of *MTHFR* variants in hematologic malignancies other than ALL.

End Point and Genetic Model. The main end point was the risk of ALL, including both adult and childhood ALL. However, sensitivity analyses were done considering adult and childhood ALL separately. A recessive model for the C677T (TT versus CT + CC) and the A1298C (CC versus AC + AA) polymorphism was assumed in line with previous research (9). However, pairwise comparisons between 677TT versus 677CC and 1298CC versus 1298AA genotypes were also done.

Data Extraction and Statistical Analysis. The characteristics of the included studies were independently extracted by two investigators (T.V.P. and M.R.) through a standardized protocol. Results were compared and minor disagreements were resolved by discussion and rereading of the original data.

The odds ratio (OR) was used as the metric of choice for the evaluation of risk. We combined OR and its 95% confidence intervals (95% CI) using both fixed-effects (15) and random-effects (16) models according to the Mantel-Haenszel and DerSimonian-Laird methods, respectively. The Cochran's Q-statistic test was used to assess the presence of heterogeneity (16). To evaluate the robustness of the overall estimate, a sensitivity analysis was done by iteratively eliminating each individual study and recalculating the summary OR. Sensitivity analysis was also done considering adult and childhood ALL separately. In addition, we carried out a further sensitivity analysis including studies in which the controls were not in accordance with the Hardy-Weinberg equilibrium (14). For such analysis, the ORs and the variances of studies with deviations from the Hardy-Weinberg equilibrium were

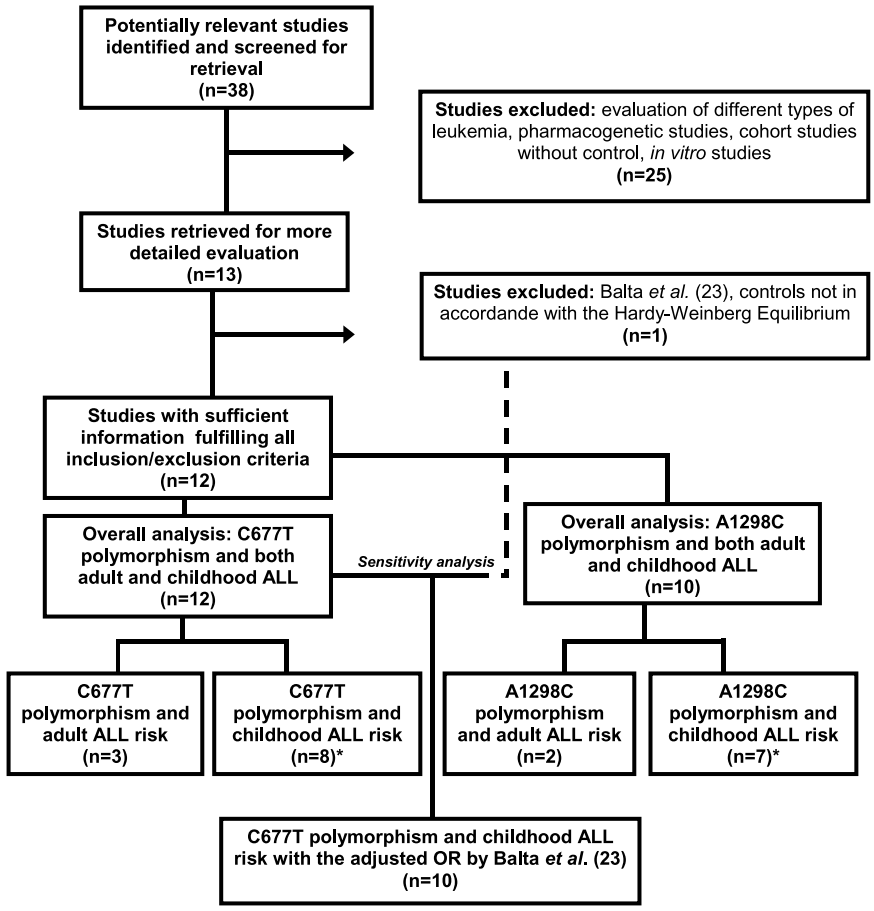

Figure 1. Flow chart outlining the study selection process for the effect of MTHFR variants on the risk of ALL. Note that the study by Chiusolo et al. (25) was only included in the overall analysis (both adult and childhood combined) due to the impossibility of stratifying cases by leukemia type.

adjusted, as previously reported (17). Details and applications of the above method are described elsewhere (14). Evidence for publication bias was assessed using the Egger's regression asymmetry statistics (18) and the Begg-Mazumdar adjusted rank correlation test (19). A meta-regression analysis was done to delineate possible reasons for heterogeneity among studies. The OR was the dependent variable, whereas year of publication, total sample size, and journal impact factor (according to the Journal of Citation Reports 2004) were independent variables. Hardy-Weinberg equilibrium was tested for controls using an exact test. A P value of <0.05 was judged significant with exception of the Q-statistic, in which a significance level of <0.1 was chosen. All analyses were done with the Stata statistical package (version 7.0; College Station, TX).

Results

Study Selection and Overview of the Studies Characteristics. A diagram flow chart summarizing the process of study selection is depicted in Fig. 1. The final search generated 13 potentially relevant studies (20-32) in which the effect of *MTHFR* polymorphisms on the risk of both adult and childhood ALL was evaluated. Contacts to experts in the field failed to indicate the presence of any unpublished study. Among the 13 published studies retrieved, 12 were full-length reports (20-28, 30, 32) and 1 was a letter (31). Of these, seven studies assessed the effect of *MTHFR* variants in populations of European descent (25-31), three studies evaluated populations predominantly (~90%) composed by European descendants (20-22), two studied populations from Turkey (23, 24), and

Table 1. Characteristics of studies included in the meta-analyses

Study	Year	Country	Setting	Period of case diagnosis
Skibola et al. (20)	1999	United Kingdom	Cases selected from various centers of United Kingdom. Controls selected randomly from a list of all patients registered with the same local physician. Matching factors: center, gender, age and ethnicity	April 1991-December 1996
Franco et al. (21)	2001	Brazil	Single center. Controls selected from the same center. Matching factors: age, gender, and ethnicity	January 1991-January 2000
Wiemels et al. (22)	2001	United Kingdom	Cases selected from several centers of United Kingdom. Cases ages <15 y. Controls were healthy, newborn infants	1992-1998
Balta et al. (23)	2003	Turkey	Cases selected from two centers of Turkey. Controls consisted of randomly selected, unrelated, healthy individuals without historical of malignancy	February 2000-February 2002
Deligezer et al. (24)	2003	Turkey	Not described	Not described
Chiusolo et al. (25)	2004	Italy	Cases from central Italy. Controls were blood donors without family history of hematologic diseases	From 1995
Gemmati et al. (26)	2004	Italy	Cases were recruited in two centers in Italy. Controls consisted of healthy individuals from the same areas randomly selected from blood donor lists	January 1990-December 2001
Krajinovic et al. (27)	2004	Canada	Single center. Controls were healthy individuals from diverse clinical departments	August 1988-May 2001
Chatzidakis et al. (28)	2005	Greece	Two centers in Greece. Cases were 52 consecutive ALL patients. Controls were 88 adults with no history of malignancies	1997-2004
Oliveira et al. (29)	2005	Portugal	Two centers in Portugal. Cases were children ages <16 y. Control group consisted of unrelated healthy individuals born in the same geographic region with no history of malignant diseases	Not described
Schnakenberg et al. (30)	2005	Germany	84 different treatment centers in Germany, Austria, and Switzerland	July 1999-February 2001
Thirumaran et al. (31)	2005	Germany	Not described	1983-2003
Zanrosso et al. (32)	2006	Brazil	Cases selected from various centers covering the main regions of Brazil. Controls were healthy subjects from the same regions	Not described

Abbreviation: AML, acute myelogenous leukemia.

one examined both mixed and European-derived populations (32). Genotypic data for both C677T and A1298C variants were available for 10 studies (20-22, 25-32), whereas 3 reports presented only data for the C677T polymorphism (23, 24, 28). However, one study (23) was further excluded from the main analysis due to lack of agreement of controls with the Hardy-Weinberg equilibrium ($P = 0.027$, exact test). Altogether, a total of 12 references (13 subgroups) fulfilled all inclusion criteria. Among the 13 subgroups assessing the C677T variant, 8 reported a statistically significant association between the 677T allele (or 677TT genotype) and a reduced risk of ALL. For the 10 subgroups investigating a link between the A1298C variant and the risk of ALL, only 3 suggested a statistically significant protective effect for the 1298C allele (or 1298CC genotype) on ALL risk, whereas 1 reported statistically significant results in an opposite direction (32). Details of the characteristics of these studies and their main results are summarized in Table 1.

C677T Polymorphism and the Overall Risk of ALL: Evidence for Publication Bias and Reduced Risk Only for Adult ALL. The main meta-analysis of the association between the C677T polymorphism and risk of ALL included 13 subgroups from 12 studies (20-22, 24-32) with a total of 5,628 subjects (2,191 cases and 3,437 controls). The control groups were hospital inpatients, community controls, or blood donors without history of malignant diseases (Table 1).

There was no statistically significant evidence for heterogeneity of the ORs among the 13 case-control subgroups in a recessive model (TT versus CT + CC, Q-statistic, $\chi^2 = 16.28$, degrees of freedom (df) = 12, $P = 0.18$). The summary OR under a fixed-effects model showed that subjects homozygous

Table 1. Characteristics of studies included in the meta-analyses (Cont'd)

Leukemia characteristics	Main results	Hardy-Weinberg equilibrium
Adult ALL. Immunophenotype: 71% B-ALL, 15% T-ALL, and 14% undetermined	677TT genotype associated with a significantly decreased risk of ALL (TT vs CC, OR, 0.23; 95% CI, 0.06-0.81). 1298AC genotype was related to a 3-fold reduction in risk of ALL (OR, 0.33; 95% CI, 0.15-0.73)	Yes
Childhood ALL. Immunophenotype: 77% B-ALL and 23% T-ALL	677T carries associated with a significantly decreased risk of ALL (TT + CT vs CC, OR, 0.4; 95% CI, 0.2-0.8)	Yes
Defined molecular childhood leukemia including AML and ALL. Among the ALL cases, 36% were *TEL-AML1* and 64% were hyperdiploid leukemias	The 677T allele (TT + CT vs CC) was associated with a reduced risk of mixed-lineage leukemias (both AML + ALL included, OR, 0.36; 95% CI, 0.15-0.85), whereas the 1298CC genotype was related to a decreased risk of hyperdiploid leukemias (OR, 0.26; 95% CI, 0.07-0.81)	Yes
Childhood ALL. Immunophenotype: 52% B-ALL, 28% non-B-ALL, and 20% undetermined	No statistically significant association between ALL and the C677T *MTHFR* variant	No
Adult ALL. Immunophenotype not described	No statistically significant association between ALL and the C677T *MTHFR* polymorphism	Yes
Both Adult and childhood ALL. Immunophenotype not described	No statistically significant association between ALL and both the C677T and A1298C *MTHFR* polymorphisms	Yes
Adult ALL. Immunophenotype: 73.3% B-ALL, 16.6% T-ALL, and 10% undetermined	677T carriers associated with a statistically significant decrease on the risk of ALL. No statistically significant association between ALL and the A1298C *MTHFR* polymorphism	Yes
Childhood ALL. Immunophenotype: 85% pre B-ALL, 11% T-ALL, and 4% undetermined	Reduced risk of childhood ALL for the 677TT genotype only in children born before 1996. The TT677/AA1298 and CC677/CC1298 individuals were associated with reduced risk of ALL (OR, 0.4; 95% CI, 0.2-0.9; and OR, 0.3; 95% CI, 0.1-0.6, respectively)	Yes
Childhood ALL. Immunophenotypes: 87% B-ALL and 13% T-ALL	Reduced risk for developing ALL in 677T allele carriers (TT + CT vs CC, OR, 0.39; 95% CI, 0.19-0.78). Protective effect of the 677T allele accentuated in the B-ALL group (TT + CT vs CC, OR, 0.31; 95% CI, 0.15-0.66)	Yes
Childhood ALL. Immunophenotype not described	No statistically significant association between ALL and both the C677T and A1298C *MTHFR* polymorphisms	Yes
Childhood ALL. Immunophenotypes: 77.7% B-ALL,18.3% T-ALL, 0.9% biphenotype, and 3.2% undetermined	No significant associations between the *MTHFR* variants and risk of ALL were observed	Yes
Not described	No statistically significant association between ALL and both the C677T and A1298C *MTHFR* polymorphisms	Yes
Childhood ALL. Immunophenotype: 94.9% B-ALL and 5.1% T-ALL	677T allele carriers (TT + CT vs CC) were associated with a decrease of ALL risk in children defined as ''non-white'' (OR, 0.43; 95% CI, 0.22-0.86). In this same group, the 1298C allele (CC + AC vs AA) was linked to an increased risk of ALL (OR, 2.93; 95% CI, 1.22-7.08)	Yes

for the 677T allele (TT versus CT + CC) were associated with a modest overall reduction in the risk of ALL (Mantel-Haenszel common OR, 0.83; 95% CI, 0.70-0.99; $P = 0.03$). As the between-study variance was not large, use of a random-effects model yielded similar results (DerSimonian-Laird common OR, 0.79; 95% CI, 0.64-0.99; $P = 0.04$). However, whereas Begg-Mazumdar test was not significant ($P = 0.32$), the Egger test indicated a putative presence of publication bias (intercept = −1.53; 95% CI, −2.80 to −0.26; $P = 0.02$). According to Egger et al. (18), this nonzero intercept suggests the possibility that small studies showing no effect of the C677T variant on ALL risk were suppressed from publication.

Thus, despite these initial positive findings, such marginal associations and the putative presence of publication bias suggest extreme caution when interpreting these results. To investigate differential effects of the TT genotype on the risk modulation for ALL, we carried out a sensitivity analysis, separately considering adult ALL and childhood ALL.

Surprisingly enough, by combining data of eight studies (21, 22, 27-32) totaling 4,567 subjects (1,772 cases and 2,795 controls), the TT genotype was not associated with a reduced risk of childhood ALL (Mantel-Haenszel common OR, 0.87; 95% CI, 0.72-1.06; $P = 0.17$). Virtually identical results were obtained when contrasting TT versus CC genotypes. Indeed, individuals homozygous for the 677T allele failed to show a reduced risk of childhood ALL when compared with CC subjects (Mantel-Haenszel common OR, 0.84; 95% CI, 0.69-1.03; $P = 0.10$). In both cases, the test of heterogeneity provides evidence for homogeneity among study results (Q-statistic, $\chi^2 = 8.27$, $df = 8$, $P = 0.41$ and $\chi^2 = 11.37$, $df = 8$, $P = 0.18$, respectively).

Interestingly, the TT genotype was associated with a significant reduction in the risk of development of adult ALL (Mantel-Haenszel common OR, 0.45; 95% CI, 0.26-0.77; $P = 0.004$), showing homogeneity among study results (Q-statistic, $\chi^2 = 1.28$, $df = 2$, $P = 0.53$). Pooled OR for the pairwise comparison (TT versus CC) rendered similar results, indicating that the TT genotype was associated with a significant reduction in the risk of development of adult ALL (Mantel-Haenszel common OR, 0.41; 95% CI, 0.24-0.72; $P = 0.002$). Homogeneity among study results was also observed (Q-statistic, $\chi^2 = 2.85$, $df = 2$, $P = 0.24$).

We also investigated the influence of a single study on the overall results. For childhood ALL, the summary ORs were computed, omitting one study at a time in a recessive model. Except after the exclusion of the largest study (31), omission of other studies generated little or no difference on the overall results. Furthermore, identical findings were obtained in the comparison between the TT and CC genotypes, suggesting robustness of our results (data not shown).

Finally, as there is no consensus on the inclusion of studies in which the controls are not in Hardy-Weinberg equilibrium (14), we carried out a last sensitivity analysis including the adjusted OR (17) by Balta et al. (23), yielding a meta-analysis of 4,894 subjects (1,914 cases and 2,980 controls). After adjustment for deviations from the Hardy-Weinberg equilibrium (17), no significant changes on the summary OR for childhood ALL were observed both in a recessive model (TT versus CT + CC: Mantel-Haenszel common OR, 0.88; 95% CI, 0.73-1.06; $P = 0.18$) and in the pairwise comparison TT versus CC (Mantel-Haenszel common OR, 0.85; 95% CI, 0.70-1.04; $P = 0.12$). No evidence for heterogeneity was detected (data not shown). A forest plot showing the summary OR on the C677T polymorphism and the overall risk of ALL (all 13 studies included), as well as pooled estimates by leukemia type, is depicted in Fig. 2.

Current Evidence Does Not Support a Reduced Risk of Acute Lymphoblastic Leukemia for the 1298CC Genotype. Ten studies with 11 subgroups totaling 5,260 subjects (2,067 cases and 3,193 controls) that evaluated the association between the A1298C polymorphism and risk of ALL were included in the second meta-analysis. As for the C677T variant, the control groups were selected mainly from hospital-based blood donors or general community populations. Egger test ($P = 0.67$) and Begg-Mazumdar test ($P = 0.93$) suggest no evidence for publication bias in this set of studies. However, Q-statistic reveals the presence of mild heterogeneity between study estimates ($\chi^2 = 18.83$, $df = 10$, $P = 0.04$). By the use of a random-effects model, no evidence for a protective effect of the CC genotype on risk of ALL was observed (CC versus CA + AA; DerSimonian-Laird common OR, 0.83; 95% CI, 0.60-1.15; $P = 0.28$). By meta-regression, none of the study-level covariates evaluated was significantly associated with the magnitude of the OR (data not shown). For sensitivity analysis, calculation was further done separately for adult ALL (two studies: 183 cases and 371 controls) and childhood ALL (eight subgroups: 1,710 cases and 2,712 controls).

Summary ORs under a random-effects model indicated no significant reduction in risk for both adult and childhood ALL for the CC genotype (DerSimonian-Laird common OR, 0.52; 95% CI, 0.05-5.03; $P = 0.57$; and DerSimonian-Laird common OR, 0.80; 95% CI, 0.56-1.16; $P = 0.24$ for adult and childhood ALL, respectively). The tests of heterogeneity for both adult ALL ($\chi^2 = 4.38$, $df = 1$, $P = 0.04$) and childhood ALL ($\chi^2 = 14.23$, $df = 7$, $P = 0.05$) were significant, indicating the presence of mild heterogeneity. Additional sensitivity analysis, in which the combined estimate of OR was computed after omission of one study at a time, reveals that the pooled estimates remain virtually the same for the childhood leukemia studies, suggesting that no single study is heavily influencing the summary OR in this meta-analysis (data not shown).

No differences comparing the 1298CC genotype with subjects homozygous for allele A (CC versus AA model) were observed. Overall, 1298CC individuals were not associated with a reduced risk of ALL when compared with the AA genotype (DerSimonian-Laird common OR, 0.86; 95% CI, 0.59-1.25; $P = 0.42$). Again, evidence for heterogeneity was observed ($\chi^2 = 26.63$, $df = 10$, $P = 0.01$). Stratification by leukemia type indicates also a lack of association between the CC genotype and a reduced risk of both childhood ALL (DerSimonian-Laird common OR, 0.83; 95% CI, 0.55-1.25; $P = 0.38$) and adult ALL (DerSimonian-Laird common OR, 0.46; 95% CI, 0.03-7.46; $P = 0.58$) when compared with the AA genotype. Meta-regression analyses failed to show a significant association between the magnitude of OR (CC versus AA) and study-level covariates (data not shown). Figure 3 summarizes graphically the overall risk of ALL, as well as pooled estimates by leukemia type, for the CC genotype.

Discussion

Whereas one may argue that the role of the A1298C polymorphism cannot be excluded in other ethnical populations and might be evident only in populations with low folate intake (27), we cannot rule out the possibility that such variant is nonfunctional. In fact, we also previously showed a lack of association between the A1298C genotypes and serum folate, serum cobalamin, or total homocysteine specifically in a mild folate-deficient Brazilian population (9). Indeed, despite some inverse positive associations (33), most current available data suggest no evidence for a major role of this variant in folate status (34) or homocysteine levels (9, 35) in populations usually characterized by mild folate deficiency. Given the presence of heterogeneity among study results and the effect in an opposite direction for the A1298C variant found in some reports (22, 32), we suggest that no additional studies are needed on this postulated association, at least for populations with adequate folate intake (30, 31).

A link between a reduced risk of childhood ALL and the C677T polymorphism is attractive due to the interesting

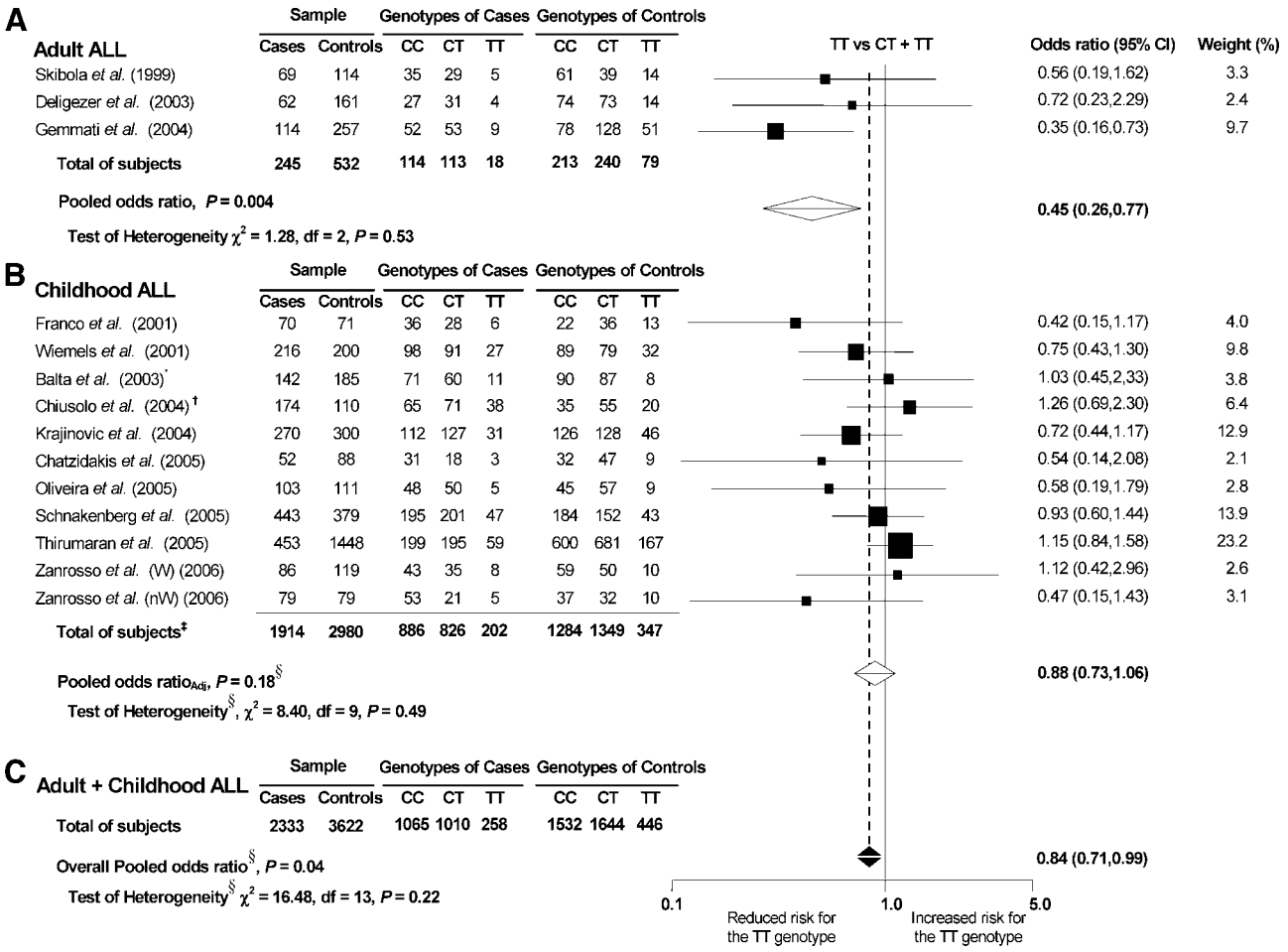

Figure 2. Association between the C677T polymorphism and risk of ALL development. Pooled OR for the 677TT genotype (TT versus CT + TT) is shown under a fixed-effects model (Mantel-Haenszel method). **A,** combined estimate for adult ALL. **B,** combined estimate for childhood ALL. **C,** overall combined estimate. *, the OR and 95% CI by Balta et al. (23) are represented by the adjusted (*Adj*) estimates (17). †, the study by Chiusolo et al. (25) was included only in the overall combined estimate. ‡, sample size of Chiusolo et al. (25) not included. §, derived using the adjusted estimates by Balta et al. (23). Square sizes are proportional to weight of each study in the meta-analysis. Weights are given for the overall combined OR. *W,* White; *nW,* non-White, as reported by the authors (32).

biological mechanism, particularly because this variant has been found to be unequivocally associated with folate and homocysteine levels (9). However, through the combined examination of 4,567 subjects, the present meta-analysis does not support a protective effect of the 677TT genotype on a reduced risk of pediatric ALL. Importantly, these results were extremely robust to different model assumptions (fixed-effects versus random-effects models), genetic models, and additional sensitivity analysis. Furthermore, the pooled estimate was derived from a set of homogenous studies, which does not support the hypothesis that this polymorphism may have an effect in some populations but not in others.

What then is the explanation for this lack of association? Actually, several factors may underlie the absence of an association between childhood ALL and the C677T polymorphism.

First, as previously mentioned, single variants are unlikely to be responsible for complex hematologic malignancies, where an unfavorable gene-environment interaction is likely to play a major role (20). Because none of the 13 genetic association studies included in the present meta-analysis was also designed to investigate the interaction between folate status or maternal folate intake during the pregnancy and the risk of ALL, conflicting results from various countries may reflect the complex interaction between genetic variants in the folate pathway and nutrient intake.

Second, the *MTHFR* C677T and A1298C variants are 2.1 kb apart and have indicated to be in strong linkage disequilibrium (5). In this respect, specific haplotype frequencies could be also a source of conflicting results. Hence, haplotypic analysis and gene-gene interactions with other genes coding for important enzymes in folate metabolism are research gaps to be filled, requiring further exploration.

Third, previous evidence suggests a more prominent role for the maternal *MTHFR* genotype and folate status in the risk of Down syndrome (36) and cleft lip/palate (37). By analogy, another possible explanation for the negative findings between the C677T variant and a reduced risk of pediatric acute leukemia is the fact that any risk modulation in childhood ALL might depend more on both mother's genotype and altered maternal folate status during pregnancy than the child's genotype per se. Nonetheless, a recent study testing the interaction between maternal genotype and folate supplementation during pregnancy, in a homogenous cohort characterized by the same genetic and geographic background, does not support a folate-related gene-environment interaction in the etiology of childhood ALL (38). Therefore, the role of a gene-nutrient interaction for the development of childhood ALL is still uncertain, and further larger investigations assessing both the mother's genotype and folate status in the risk of pediatric ALL risk are warranted (38).

Fourth, we should also consider the phenomena of spurious association and publication bias. Indeed, empirical observations suggest that the first studies in genetic association studies commonly have small sample sizes and tend to give more impressive estimates of effect size than subsequent research (39). Furthermore, publication bias likely affects strongly the available literature, leading to overestimated genetic effects. Thus, the raised question about a false-positive association disclosed in the pioneers studies of *MTHFR* variants and risk of childhood ALL followed by publication bias cannot be ruled out. Finally, one may argue that our meta-analysis failed to have sufficient power to detect an OR of 0.8 or higher on the relation between the C677T variant and risk of childhood ALL. In fact, it must be emphasized that the power and quality of a meta-analysis depend on the size and quality of the studies being combined. Hence, the present meta-analysis has several limitations that should be acknowledged, especially the reduced number of available studies and the limited sample size of each included study. However, the pooled OR obtained for childhood ALL risk is in fully accordance with previous evidence showing that the relative risk of disease susceptibility for single genetic variants is moderate to low, most of them conferring relative risks near 1.0 (39). Thus, in a scenario where the C677T polymorphism has a real modest effect (nearing a relative risk of 1.0), the benefit of this polymorphism for clinical practice or public health remains questionable in populations with adequate folate intake for pediatric ALL, because adequate ingestion of folate may be sufficient to overcome any potential detrimental effects of *MTHFR* variants (5).

More conceivable would be the relation between the folate status and genotype in adult ALL, where folate status might be much more conditional to the subject's own genotype and folate intake. In this scenario, a role of the C677T polymorphism in risk modulation for adult ALL could be more prominent, particularly in populations with chronic folate deficiency. In fact, subgroup analyses were done and a positive association between the C677T polymorphism and the risk of adult ALL was observed. The present meta-analysis suggests a 2-fold risk reduction in adult ALL for the TT genotype when compared with the CT + CC genotypes. A similar result was also obtained when TT subjects were compared with those homozygous for the C677 allele (TT versus CC: common OR, 0.41). Although these findings were derived from a homogenous set of studies, heterogeneity tests are underpowered in meta-analysis and can reject the hypothesis of heterogeneity when the number of studies is small (18, 19). Thus, another limitation of the present meta-analysis is the reduced number of studies on the relation of adult ALL and *MTHFR* variants (three studies, 777 subjects: 245 cases and 532 controls). In addition, the extent of publication bias is unknown. Despite these drawbacks, the studies on the relation between the *MTHFR* C677T and adult ALL risk are interesting and quite consistent, deserving additional investigations.

In conclusion, current published studies fail to support the hypothesis that the *MTHFR* A1298C polymorphism is

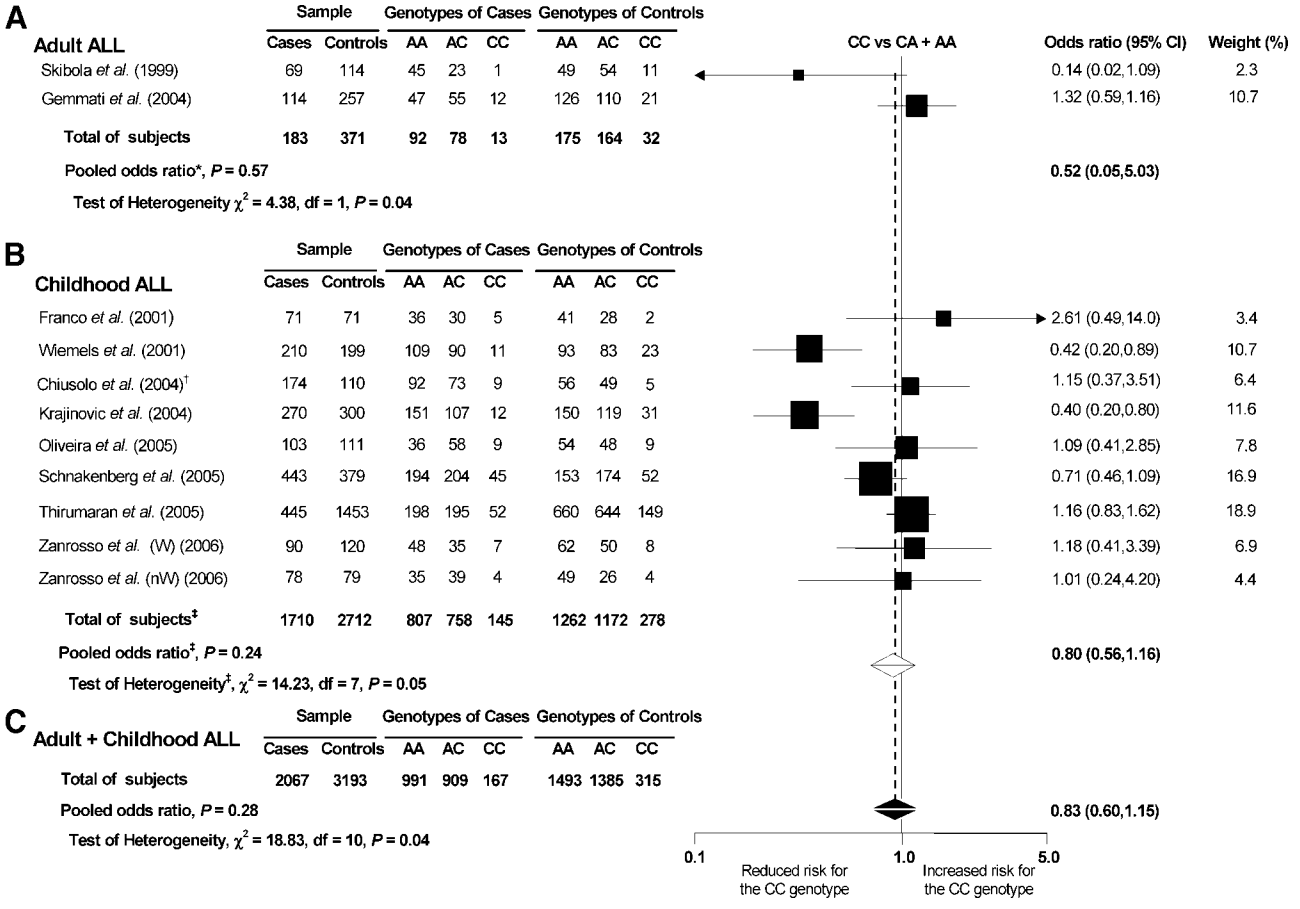

Figure 3. Association between the A1298C variant and risk of ALL development. Pooled OR for the 1298CC genotype (CC versus CA + AA) is shown under a random-effects model (DerSimonian-Laird method). **A,** combined estimate for adult ALL. **B,** combined estimate for childhood ALL. **C,** overall combined estimate. *, diamonds for the pooled estimate are not shown due to the graphical adequacy. †, included only in the overall combined estimate. ‡, data from Chiusolo et al. (25) not included. Square sizes are proportional to weight of each study in meta-analysis. Weights are given for the overall combined OR. *W,* White; *nW,* non-White, as reported by the authors (32).

associated with a reduced risk of ALL. In addition, the C677T variant does not seem to play a major role in risk modulation in pediatric ALL, at least for populations with adequate folate intake (30, 31). Further studies assessing folate level measurements to confirm any gene-environment interaction in adult ALL development are required.

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