A meta-analysis of genotypes and haplotypes of methylenetetrahydrofolate reductase gene polymorphisms in acute lymphoblastic leukemia

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Abstract. A meta-analysis of case–control studies that investigated the association between the C677T and/or A1298C polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene and acute lymphoblastic leukemia (ALL) was carried out. Pooled odds ratios (OR) of various genetic contrasts of each polymorphism were estimated using random (RE) and fixed effects (FE) models. Pooled ORs for combined genotypes and haplotypes were estimated after adjustment for study effect using a log-linear model and the expectation–maximization algorithm in combination with log-linear modeling, respectively. The recessive model for allele 1298C produced a rather marginal association: RE OR: 0.67; 95% confidence interval (CI): 0.46–0.99 and FE OR: 0.64; 95% CI: 0.49–0.84. In Caucasians, the results of the recessive model for allele 1298C was consisted with a protective effect of ALL development: FE OR: 0.63; 95% CI: 0.46–0.87. In childhood ALL, according to the results of the allele contrast and the recessive model for 677T allele it was conceivable that a protective effect exist: RE OR = 0.74; 95% CI: 0.57–0.96 and RE OR: 0.69; 95% CI: 0.51–0.94, respectively. The combined genotypes produced significant pooled OR for the 677CC/1298CC relative to 677CC/1298AA (OR: 0.54; 95% CI: 0.36–0.80). The haplotype 677C/1298C might be more protective to ALL relative to haplotype 677C/1298A (OR: 0.77; 95% CI: 0.61–0.97). When studies not in Hardy–Weinberg equilibrium (HWE) were corrected to account for departures from HWE, then, the pattern of results remained the same. Overall, there is high heterogeneity between the studies in both polymorphisms. A differential magnitude of effect in large versus small studies and alteration of early extremes effects existed.

Key words: Genotypes; Haplotypes; Hardy–Weinberg equilibrium; Leukemia; Log-linear model; Meta-analysis; MTHFR

Introduction

Little is known about the biologic mechanisms and etiology of acute lymphoblastic leukemia (ALL) which is the most common pediatric cancer accounting for 25%–30% of all childhood malignancies [1, 2]. The wide spectrum of molecular diversity of ALLs indicates that the genesis of these tumors likely involves a complex interaction between inherited predispositions, exposures to exogenous agents with leukemogenic potential and impaired hematopoietic development [3, 4].

Folate is an essential nutrient for nucleotide synthesis. Folate deficiency has been shown to induce DNA damage through uracil misincorporation into DNA during replication, leading to an increased risk of DNA double strand breaks during DNA excision repair and subsequent genetic instability [5, 6]. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and directs the flux of intracellular folate toward the conversion of homocysteine to methionine at the expense of nucleotide synthesis [7].

Two common genetic polymorphisms, C677T and A1298C, have been described for this enzyme. C677T transition is a common mutation in the coding region of the MTHFR gene which causes an alanine to valine (Ala222Val) amino acid substitution [8]. The homozygous 677TT genotype, which occurs in approximately 10%–15% of Caucasian and Asian populations [9], has been shown to have 30% of the MTHFR wild type enzyme activity in vitro, and the heterozygous 677CT genotype has approximately 60% of wild type enzyme activity [8]. Increased folic acid intake is required to maintain homocysteine remethylation to methionine at normal levels. Reduced enzyme activity of MTHFR, resulting in an intracellular accumulation of S-adenosylhomocysteine (SAH) and increase in plasma homocysteine levels...
can be associated with premature cardiovascular disease [11], neural tube defect [12], osteoporosis [13, 14] and with psychiatric conditions [15]. Another MTHFR polymorphism has been identified at position A1298C (Glu429Ala) [16]. The I298C allele has also been found to result in decreased in vitro activity and about 5%–10% of Caucasians carry the I298CC genotype [4, 17]. A1298C in combination to C677T may be associated with decreased MTHFR activity and increased homocysteine levels [18].

These polymorphic variants of MTHFR have been linked to a decreased risk of adult and pediatric ALL. This protective effect may be due to the greater availability of 5,10-methylenetetrahydrofolate and thymidine pools and to an increased fidelity of DNA synthesis [19]. The case-control studies that had investigated so far the association between ALL and the C677T and A1298C polymorphisms provided controversial or non-conclusive results. Each one typically involved a small number of cases and controls and the interpretation was complicated by the fact that different populations and sampling strategies were used.

Therefore, a meta-analysis of all available studies relating the C677T and A1298C polymorphisms of the MTHFR gene to the risk of developing ALL was carried out. In the meta-analysis the effect of allele contrast, the contrast of homozygotes, and the contrast for the dominant and recessive models were estimated. In addition, the consistency of genetic effects across populations from different racial descent, the heterogeneity between studies, and the existence of potential bias were investigated.

**Methods**

**Selection of studies**

All studies that investigated the association of the C677T and A1298C polymorphisms in the MTHFR gene with the development of ALL, published before February 2006, were considered in the meta-analysis. These studies were identified by extended computer-based search of the PubMed database. The combination of the following terms was used as a search criterion: “MTHFR”, C677T”, ”A1298C”, “acute lymphoblastic leukemia”, “leukemia”, or “ALL”.

The retrieved publications were then read in their entirety in order to assess their appropriateness for inclusion in this meta-analysis. All references cited in the studies were also reviewed to identify additional published work not indexed by PubMed database. Abstracts, case reports, editorials and review articles were excluded. The search was restricted to articles in English.

Case–control studies that determined the distribution of the C677T and/or A1298C genotypes in cases with ALL, and in controls free of malignancies were eligible for inclusion in the meta-analysis. Genome scans were excluded since they investigate linkage [20, 21] and also family-based studies association studies were not considered because of different design considerations.

**Data extraction**

From each study the following information was extracted: first author, year of publication, racial descent of study population, demographics, matching, validity of the genotyping method, and the number of cases and controls for each C677T or A1298C genotype. In addition, it was recorded whether the genotypic data were read blind in the case-control status. The frequencies of the alleles were calculated, for the cases and the controls, from the corresponding genotype distributions.

**Meta-analysis**

The meta-analysis examined the overall association of T allele with the risk of ALL relative to the C allele, and the contrast of homozygotes, the recessive model for T allele and the dominant model for T allele. The same contrasts were investigated for the C allele of the A1298C polymorphism. All associations were indicated as odds ratios (OR) with the corresponding 95% confidence interval (CI). Then, based on the individual ORs, a pooled OR was estimated.

The heterogeneity between studies was tested using the Q-statistic, which is a weighted sum of squares of the deviations of individual study OR estimates from the overall estimate [20, 21]. If $p < 0.10$ then the heterogeneity was considered statistically significant. Heterogeneity was quantified with the $I^2$ metric, which is independent of the number of studies in the meta-analysis [22]. $I^2$ takes values between 0% and 100% with higher values denoting greater degree of heterogeneity ($I^2 = 0\%–25\%$: no heterogeneity; $I^2 = 25\%–50\%$: moderate heterogeneity; $I^2 = 50\%–75\%$: large heterogeneity; $I^2 = 75\%–100\%$: extreme heterogeneity; when $I^2 < 0$ then $I^2 = 0$).

The pooled OR was estimated using fixed effects (FE) (Mantel-Haenszel) and random effects (RE) (DerSimonian and Laird) models. RE modeling assumes a genuine diversity in the results of various studies, and it incorporates to the calculations a between-study variance. Therefore, when there is heterogeneity between studies then the pooled OR is preferably estimated using the RE model [23]. Adjusted estimates of OR were considered whenever possible in a separate analysis. A cumulative meta-analysis [24, 25] and recursive cumulative meta-analysis were carried out in order to evaluate the trend of pooled OR for the allele contrast in time. A differential magnitude of effect in large versus small studies for the allele contrast was checked using the