Association of MTHFR C677T and A1298C Polymorphisms with Susceptibility to Chronic Lymphocytic Leukemia: A Systematic Review and Meta-Analysis

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Abstract
Background: The relation between methylenetetrahydrofolate reductase (MTHFR) polymorphisms and the risk of developing Chronic lymphocytic leukemia (CLL) is not still clear, while there are reports about the association of MTHFR C677T and A1298C polymorphisms with developing CLL, there are other reports that rolled out the association of MTHFR polymorphisms with developing CLL. Therefore herein we carried out this meta-analysis to clear the association of MTHFR polymorphisms with the risk of CLL.

Methods: A comprehensive search was performed through PubMed, Scopus and Embase from inception to Aug 2020. Odds ratios (OR) with their corresponding 95% confidence intervals (CI) for five possible genetic models were calculated. Heterogeneity was evaluated using the Cochran Q test and the I2 statistic.

Results: Totals of 1290 cases and 1887 controls for the C677T polymorphism and 1117 cases and 1256 controls for the A1298C polymorphism were included in our analysis. Analyzing the MTHFR C677T and A1298C polymorphisms genotypes showed an association between MTHFR polymorphism at A1298C under Allelic model and the risk of CLL (OR = 1.12, 95% CI = 1.01–1.25), however there was no association between MTHFR polymorphism at MTHFR C677T and risk of CLL.

Conclusion: The risk of developing CLL might be associated with MTHFR polymorphism at A1298C under allelic model and not associated with MTHFR polymorphisms at C677T. However, further studies considering other factors such as age, gender, ethnicity, gene-gene interaction and environmental condition are needed to clear the true association of MTHFR polymorphisms with CLL.

Keywords: Methylenetetrahydrofolate reductase gene; Meta-analysis; Chronic lymphocytic leukemia; Polymorphism

Introduction

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western countries, which accounts for approximately 20,720 diagnosed cases and 3930 deaths in 2019 in the United States (1). CLL is a clinically heterogeneous disease defined by the clonal proliferation and accumulation of typical mature CD5-positive B-cells within the blood, bone marrow, lymph
nodes and spleen (2). While there are advances in treatment options but it is still remained an incurable malignancy (3) with unclear etiology (4). Hence studies to identify the possible risk factors for developing CLL are in a growing interest among researchers from different fields (5). CLL is a multifactorial disorder with several risk factors including family background, race, old age and exposure to certain chemical compounds (6). Family background as risk factor for developing CLL drew the attentions toward genes and their differences between patients and healthy people; therefore nowadays research on gene polymorphisms as a genetic variation gained a much more attention among researchers around the globe to identify polymorphisms that increase the risk of developing CLL (3).

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in methionine and folate metabolism, which are involved in nucleotide synthesis and DNA methylation (7). DNA methylation plays a critical role in gene regulation and cellular differentiation (8) and its disturbance may be associated with occurrence of cancer (9). MTHFR gene is located on chromosome 1, two common polymorphisms of this gene at C677T and A1298C reported that change its enzymatic activity thus proposed that influence susceptibility to CLL (10). However results are almost inconsistent (11), therefore herein we performed this meta-analysis to reduce heterogeneity and summarize evidence about association of MTHFR C677T and A1298C polymorphisms with the risk of developing CLL.

Methods

This meta-analysis was conducted according to observational studies in epidemiology (MOOSE) guidelines and results were reported based on Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (12).

Search Strategy

A comprehensive search through PubMed, Scopus and Embase was conducted from inception to 10th Aug 2020 to identify studies reporting the association between MTHFR polymorphism and CLL susceptibility with using the following keywords; (((Methylenetetrahydrofolate reductase[Title/Abstract]) OR MTHFR[Title/Abstract])) AND (((polymorphism[Title/Abstract]) OR mutation[Title/Abstract]) OR variant[Title/Abstract])) AND ((chronic lymphocytic leukemia[Title/Abstract]) OR CLL[Title/Abstract]). Furthermore, references of all included papers were screened to find possibly eligible papers.

Eligibility criteria

The following inclusion criteria were applied on potential eligible studies to be included in this meta-analysis

1. Case–control, cohort or cross-sectional studies
2. Measure the relationship between MTHFR C677T and A1298C polymorphisms and CLL
3. The results were reported as odds ratio (OR) with corresponding 95 percent confidence interval (95% CI)
4. When same authors published two or more papers with possible same date, we used the most recent or informative paper in our meta-analysis
5. Full text published in English

Studies were excluded when they were non-English articles, books, reviews, Letters, animal studies, and comments.

Data Extraction and Quality Assessment

Two reviewers (AR and BRK) independently extracted the following data from eligible studies: The first author’s name, publication year, country, sample size, genotyping method and frequency of genotype/allele in cases and controls. Any disagreement between two reviewers was solved by third expert. The Newcastle-Ottawa Scale (NOS) for non-randomized studies was used to examine the methodological quality of included papers (13).
**Statistical analysis**

Chi-Square test was employed to calculate deviation from Hardy-Weinberg equilibrium (HWE) in control groups (14). To evaluate the association of MTHFR C677T and A1298C polymorphisms with CLL, odd ratios (ORs) with 95% confidence intervals (95% CI) were calculated for the following five genetic models for C677T polymorphism: allelic model (T vs. C), recessive model (TT vs. CT/CC), dominant model (CT/TT vs. CC), heterozygote contrast (CT vs. CC), and homozygotes contrast (TT vs. CC), and for A1298C polymorphism: allelic model (C vs. A), recessive model (CC vs. AC/AA), dominant model (AC/CC vs. AA), heterozygote contrast (AC vs. AA), and homozygotes contrast (CC vs. AA). The heterogeneity across included studies was assessed using Cochran’s Q test and I² statistics (15). Fixed-effects model was employed when the level of heterogeneity was low (P > 0.1 or I² < 50%), otherwise a random-effects model (Der Simonian–Laird approach) was used (P < 0.1 or I² > 50%) (16, 17). Sensitivity analysis (18) was done to examine the effect of each study on the final result of allelic model for MTHFR C677T polymorphism; we did not perform sensitivity analysis for MTHFR A1298C polymorphism due to limited number of studies. Egger’s linear regression test was applied to examine the publication bias (19). Analyses were performed using STATA software (version 15.0; StataCorp LLC, College Station, TX, USA) RRID: SCR_012763.

**Results**

**Search results and Characteristics of the included studies**

The primary search yielded 30 studies on the association of MTHFR polymorphisms with CLL (PubMed: 2, Scopus18, Embase: 10). Of those 22 were excluded based on duplication remove, title and abstract screening. Finally, eight studies were identified eligible to be included in our meta-analysis. There were eight studies with 1290 cases and 1887 controls for the C677T polymorphism, and 4 articles with 1117 cases and 1256 controls for the A1298C polymorphism, the detailed study flow is illustrated in Fig. 1.
The characteristics of included articles and distribution of genotypes and alleles in cases and controls are given in Table 1 and Table 2. The NOS scores for all eligible studies ranged from 6 to 8 and are shown in Table 1.

| Reference | Published year | Country | Genotyping method | Cases/Controls | Quality score |
|-----------|----------------|---------|-------------------|----------------|---------------|
| C677T polymorphism | | | | | |
| (21) | 2000 | Spain | PCR | 37/200 | 6 |
| (22) | 2004 | Germany | PCR-RLFP | 111/92 | 6 |
| (23) | 2005 | UK | PCR-RLFP | 832/886 | 7 |
| (24) | 2007 | Russia | PCR | 83/177 | 7 |
| (20) | 2008 | Serbia | PCR-RLFP | 23/35 | 6 |
| (10) | 2012 | India | PCR-RLFP | 39/251 | 8 |
| (8) | 2018 | Turkey | RT-PCR | 91/101 | 6 |
| (11) | 2019 | Brazil | PCR-RLFP | 74/145 | 7 |
| A1298C polymorphism | | | | | |
| (22) | 2004 | Germany | PCR-RLFP | 111/92 | 6 |
| (23) | 2005 | UK | PCR-RLFP | 832/886 | 7 |
| (24) | 2007 | Russia | PCR | 83/177 | 7 |
| (8) | 2018 | Turkey | RT-PCR | 91/101 | 6 |

| Reference | CLL cases | Healthy controls | P-HWE | MAF |
|-----------|------------|------------------|-------|-----|
| C677T polymorphism | | | | |
| (21) | | | | |
| (22) | | | | |
| (23) | | | | |
| (24) | | | | |
| (20) | | | | |
| (10) | | | | |
| (8) | | | | |
| (11) | | | | |
| A1298C polymorphism | | | | |
| (22) | | | | |
| (23) | | | | |
| (24) | | | | |
| (8) | | | | |

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Association between the MTHFR C677T and A1298C polymorphisms and CLL

Analyses of 8 included studies showed no significant association between MTHFR C677T polymorphism and the risk of developing CLL under five genetic models including: the dominant model; recessive model; allelic model; TT vs. CC; and CT vs. CC. Similarly, no significant association was observed in four models of MTHFR A1298C polymorphism: the dominant model; recessive model; CC vs. AA; and AC vs. AA; however there was an association between Allelic model and the risk of CLL. Results are summarized in Table 3. Moreover, the forest plots for Allelic models are given in Fig. 2 and Fig. 3.

Fig. 2: Forest plot of the association between MTHFR gene C677T polymorphism and CLL risk under Allelic model

| Study ID | Allelic Model | OR (95% CI) | Weight % |
|----------|---------------|-------------|----------|
| Gonzalez (2000) | 1.02 (0.66, 1.73) | 4.57 |
| Nuckol (2004) | 0.89 (0.59, 1.36) | 7.36 |
| Rudd (2005) | 0.97 (0.64, 1.42) | 61.98 |
| Ora (2007) | 0.83 (0.55, 1.26) | 7.47 |
| Rokanjas (2008) | 0.84 (0.28, 1.49) | 1.84 |
| Hussain (2012) | 1.45 (0.86, 2.41) | 3.67 |
| Unay (2018) | 0.76 (0.49, 1.19) | 6.39 |
| Rets (2019) | 1.33 (0.86, 2.06) | 6.72 |
| Overall (I-squared = 0.0%, p = 0.470) | 0.97 (0.86, 1.08) | 100.00 |

Table 3: Main results of pooled ORs in meta-analysis of MTHFR polymorphisms and CLL

| Genetic model | Test of association | Test of heterogeneity | Test of publication bias |
|---------------|---------------------|-----------------------|--------------------------|
| C677T polymorphism | OR 95% CI | I^2 (%) | P | T | P |
| Dominant model | 0.99 0.85-1.15 | 0.0 | 0.785 | 0.01 | 0.990 |
| Recessive model | 0.89 0.69-1.14 | 35.7 | 0.143 | -0.22 | 0.835 |
| Allelic model | 0.97 0.86-1.08 | 0.0 | 0.470 | -0.08 | 0.939 |
| TT vs. CC | 0.90 0.69-1.16 | 35.1 | 0.148 | -0.21 | 0.838 |
| CT vs. CC | 1.02 0.87-1.19 | 0.0 | 0.841 | -0.33 | 0.756 |
| A1298C polymorphism | Dominant model | 1.00 0.85-1.18 | 0.0 | 0.473 | 1.53 | 0.265 |
| Recessive model | 1.05 0.79-1.38 | 39.6 | 0.147 | 1.17 | 0.362 |
| Allelic model | 1.12 1.01-1.25 | 57.9 | 0.068 | 1.85 | 0.205 |
| CC vs. AA | 1.04 0.88-1.45 | 47.3 | 0.128 | 1.26 | 0.336 |
| AC vs. AA | 1.00 0.84-1.19 | 0.0 | 0.843 | 1.38 | 0.303 |

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Sensitivity analysis and Publication bias

Sensitivity analysis showed that pooled OR and 95% CI were not affected by omitting any single publication (Fig. 4). There was no significant heterogeneity within the included studies except for the Allelic model of the A1298C polymorphism ($I^2 = 69.8\%$). The results of Egger’s test showed no evidence of publication bias in this meta-analysis (Table 3), and the funnel plots showed no evidence of asymmetry (Fig. 5).
Discussion

To our best knowledge this was the first meta-analysis performed to explore the relationship between MTHFR gene polymorphisms and CLL risk. Overall, our result which included a high number of subjects (n = 3177 for C677T and n = 2373 for A1298C polymorphism) from medium and quality studies (e.g., NOS score ≥ 6) with no publication bias suggested that MTHFR polymorphisms at A1298C under Allelic Model is associated with the risk of developing CLL, however other genetic models and also MTHFR polymorphism at C677T might not be associated with the risk of developing CLL.

MTHFR polymorphism at C677T influence the susceptibility risk of CLL and suggested 677CC as a risk factor for developing CLL (20), however this might be due to low sample size (23cases, 35 controls). MTHFR C677T polymorphism reported to decreases the risk of CLL in recessive genetic model (P=0.03) while C allele and CC genotype of A1298C polymorphism increased the risk of CLL (OR=1.52, P=0.04; OR=6.16, P=0.005, respectively) (8). In another study, Gonzales et al reported no association between MTHFR C677T polymorphism and the risk of developing CLL in Spanish population, however they reported a protective role of 677C allele for developing Multiple Myeloma (OR=0.28, 95% CI=0.10–0.77) (21).

No relationship between MTHFR C677T and A1298C with CLL was reported in German population (22). Furthermore in a study with 832 patients and 886 healthy controls, no association between MTHFR C677T and A1298C polymorphisms and CLL was found (677TT; OR=0.90, 95% CI=0.66–1.24 and 1298CC OR=0.97, 95% CI=0.79–1.18) (23). In addition in a Russian population no association between MTHFR C677T (677TT; OR=0.6, 95% CI=0.2–1.24) and A1298C (1298CC; OR=0.9, 95% CI=0.3–2.5) with the risk of developing CLL was reported (24). Moreover in an Indian population although, a higher frequency of the MTHFR 677T allele in CLL cases was reported, no significant association between MTHFR C677T polymorphism with the risk of developing CLL was found (10). Reis al in Brazilian population found no association between MTHFR C677T polymorphism and the risk of CLL, however the TT genotype was exclusively found in men (11).

MTHFR, a 656 amino acids protein is the key enzyme of folate metabolism, which is a coenzyme involved in DNA synthesis and methylation, and its depletion has been suggested to be associated with different types of cancers (25). MTHFR convert 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and provides the methyl group for homocysteine to methionine conversion and consequently DNA methylation(26).
MTHFR mutation at C677T results in alanine to valine substitution and is associated with reduced MTHFR enzyme activity in TT genotype, by approximately 70% of the wild type (CC genotype), and MTHFR activity in CC genotype carriers of MTHFR A1298C polymorphism reduced about 40% of its wild type (AA genotype) (27). Studies reported an association between MTHFR polymorphisms and the risk of developing several types of cancer (28-31). Herein we found an association between MTHFR A1298C polymorphism and CLL under Allelic model while no association between MTHFR polymorphisms at C677T and CLL susceptibility was observed; however our results should be interpreted with caution, since the effect of gender and ethnicity on the association of MTHFR polymorphism with cancer is highlighted in several reports (8, 28).

The current meta-analysis had two major limitations: first, lack of enough information in included studies, made it impossible to examine the effect of other parameters such as age, ethnicity, gender, MTHFR polymorphisms interaction with other genes or interaction with environmental factors, second, limited number of included studies with small sample sizes. Therefore further studies with focusing on the effects of other parameters such as age, gender, ethnicity, environmental factors, and gene-gene interaction are needed to indicate the association of MTHFR polymorphisms with risk of developing CLL.

Conclusion

The risk of developing CLL might be associated with MTHFR polymorphism at A1298C under allelic model and not associated with MTHFR polymorphisms at C677T, however there are inconsistent reports about the association of MTHFR polymorphisms with the risk of developing CLL. Therefore further studies considering other parameters such as age, gender, ethnicity, gene-gene interactions and environmental condition are needed to reveal the association of MTHFR polymorphisms with CLL.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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