Iran J Public Health, Vol. 50, No.1, Jan 2021, pp.83-92

Review Article

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

Atefeh RAOUFI [1], ***Behdad RAHIMI KELARIJANI*** [1], ***Hamid Reza AHADI*** [1], ***Bahareh HASSANI DERAKHSHANDEH*** [2], ***Zahra NOOROOLLAHZADEH*** [3], ****Abbas HAJIFATHALI*** [1]

1. *Hematopoietic Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
2. *Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran*
3. *Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran*

***Corresponding Author:** Email: a.hajifathali@sbmu.ac.ir

(Received 14 Mar 2020; accepted 19 May 2020)

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 form 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 I^2 statistics (15). Fixed-effects model was employed when the level of heterogeneity was low ($P > 0.1$ or $I^2 < 50\%$), otherwise a random-effects model (Der Simonian–Laird approach) was used ($P < 0.1$ or $I^2 > 50\%$) (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.

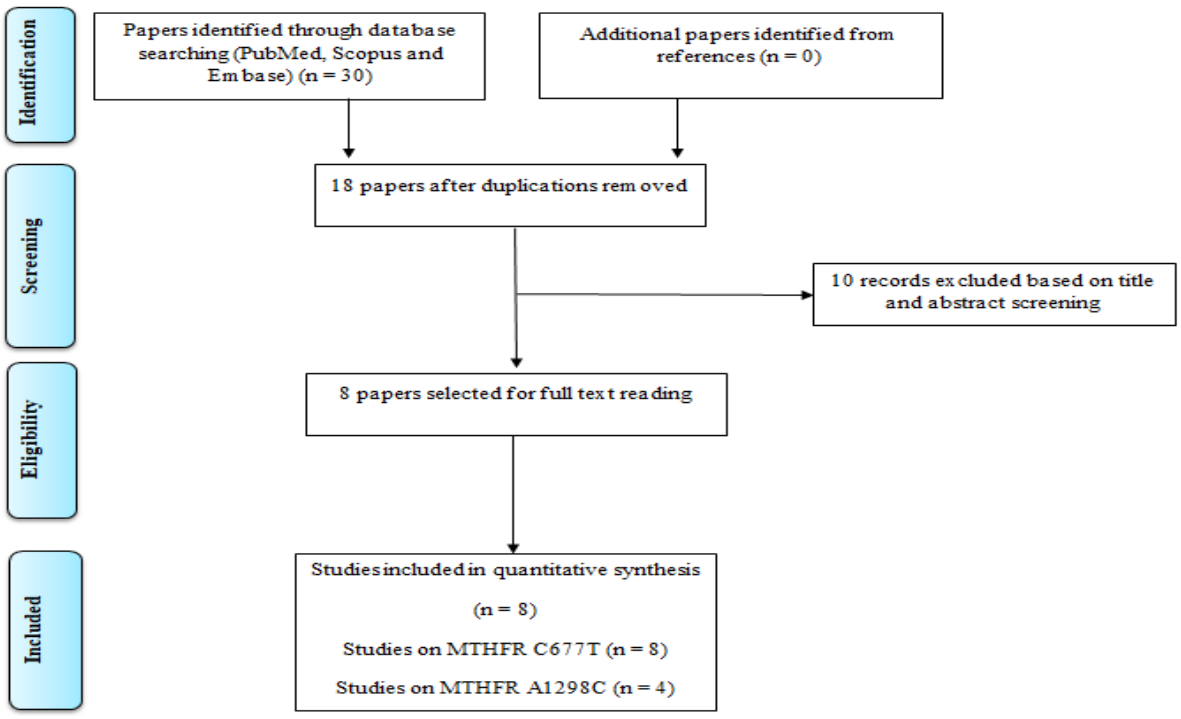

Fig. 1: Flow chart of study selection

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.

Table 1: Characteristics of the included studies

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

Table 2: Distribution of genotypes and alleles in cases and controls

Reference	*CLL cases*					*Healthy controls*						
C677T polymorphism	CC	CT	TT	C	T	CC	CT	TT	C	T	P-HWE	MAF
(21)	16	18	3	50	24	92	88	20	272	128	0.88	0.32
(22)	56	43	12	155	67	43	38	11	124	60	0.56	0.32
(23)	361	381	90	1103	561	383	397	106	1163	609	0.84	0.34
(24)	44	35	4	123	43	85	79	13	249	105	0.35	0.29
(20)	43	9	1	35	11	16	15	4	47	23	0.86	0.32
(10)	27	7	5	61	17	180	61	10	421	81	0.11	0.16
(8)	48	40	3	136	46	51	38	12	140	62	0.24	0.30
(11)	30	41	3	101	47	74	67	4	215	75	0.01	0.25
A1298C polymorphism	AA	AC	CC	A	C	AA	AC	CC	A	C	P-HWE	MAF
(22)	51	48	12	150	72	45	40	7	130	54	0.29	0.29
(23)	397	363	72	1157	507	412	389	85	1213	559	0.32	0.31
(24)	39	38	6	116	82	81	82	14	244	110	0.31	0.31
(8)	23	46	22	92	90	35	53	13	123	79	0.39	0.39

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 inTable 3. Moreover, the forest plots for Allelic models are given in Fig. 2 and Fig. 3.

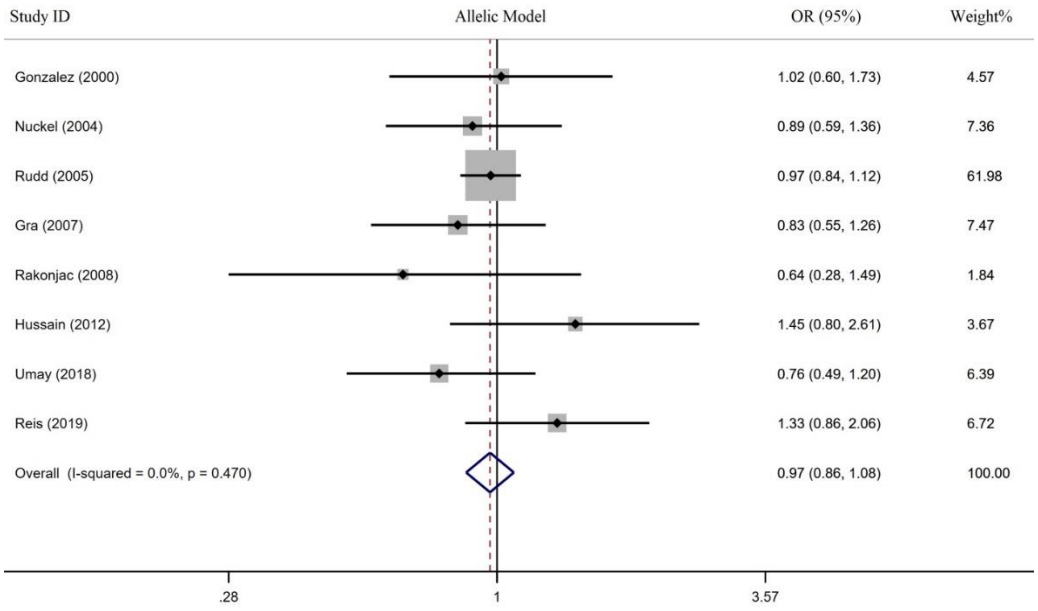

Fig. 2: Forest plot of the association between *MTHFR* gene C677T polymorphism and CLL risk under Allelic model

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

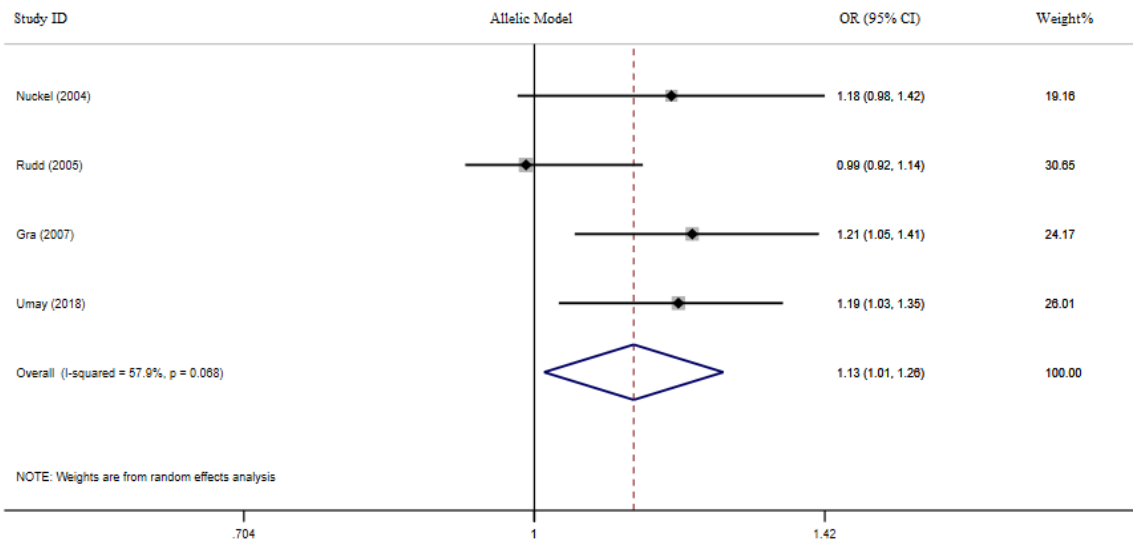

Fig. 3: Forest plot of the association between *MTHFR* gene A1298C polymorphism and CLL risk under Allelic model

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)

.

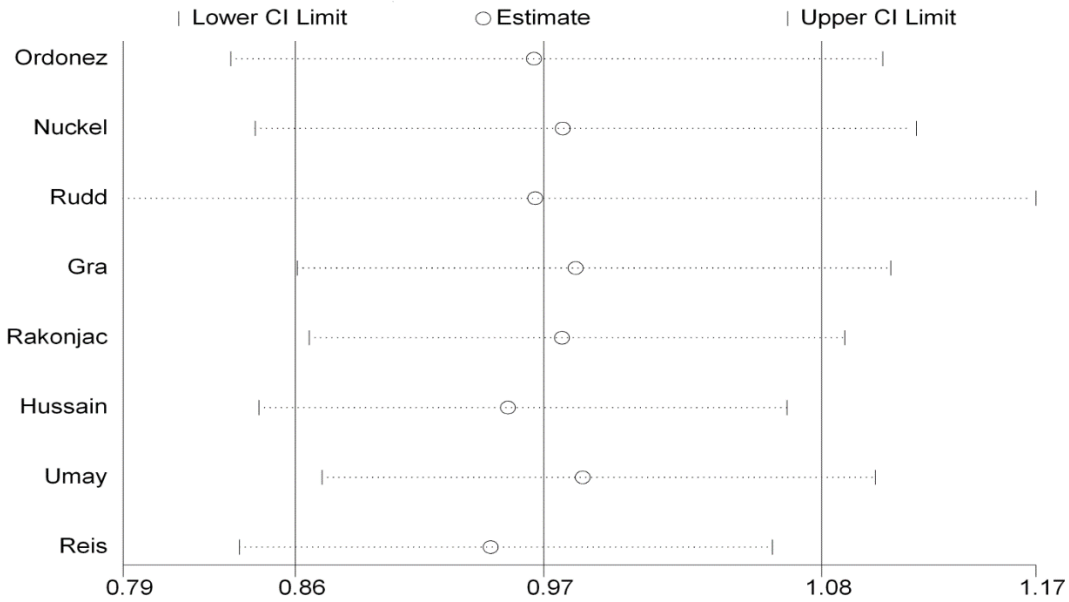

Fig. 4: Sensitivity analysis graph for included studies (given named study is omitted)

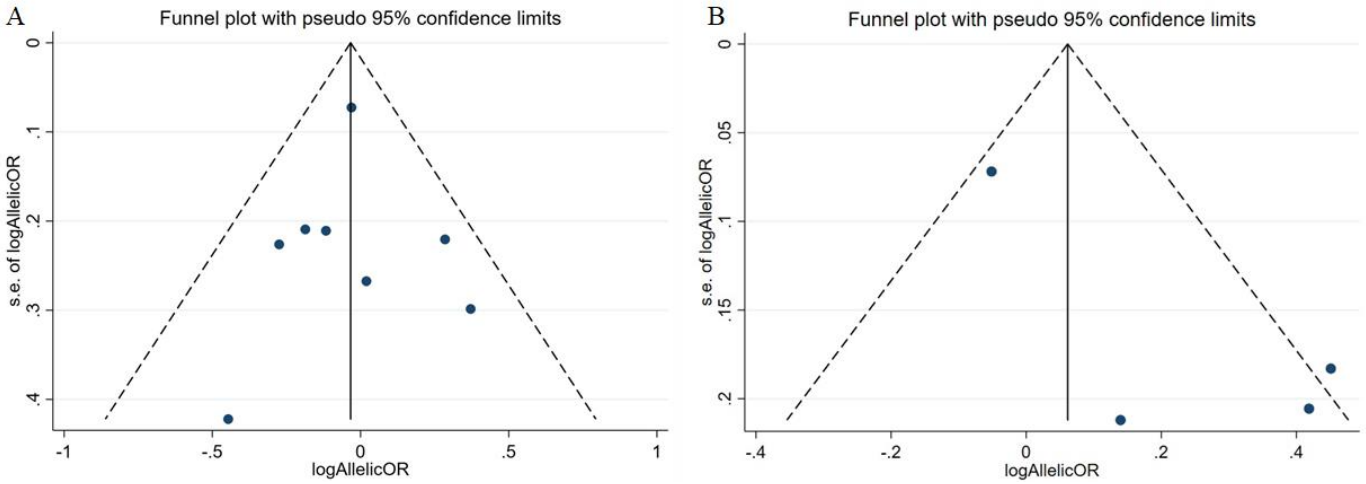

Fig. 5: Funnel plot on the association between *MTHFR* gene C677T (A) and A1298C (B) polymorphisms and CLL under Allelic model

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 et 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.

Acknowledgements

The authors received no specific funding for the research.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A (2019). Cancer statistics, 2019. *CA Cancer J Clin,* 69:7-34.
2. Hallek M (2019). Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. *Am J Hematol,* 94(11):1266-1287.
3. Berndt SI, Camp NJ, Skibola CF, et al (2016). Meta-analysis of genome-wide association studies discovers multiple loci for chronic lymphocytic leukemia. *Nat Commun,* 7:10933.
4. Oancea SC, Rundquist BC, Simon I, et al (2017). County level incidence rates of chronic lymphocytic leukemia are associated with residential radon levels. *Future Oncol,* 13(21):1873-1881.
5. Karakosta M, Delicha E-M, Kouraklis G, Manola KN (2016). Association of various risk factors with chronic lymphocytic leukemia and its cytogenetic characteristics. *Arch Environ Occup Health,* 71(6):317-329.
6. Zhou Y, Lu H, Yang M, Xu C (2019). Adverse drug events associated with ibrutinib for the treatment of elderly patients with chronic lymphocytic leukemia: A systematic review and meta-analysis of randomized trials. *Medicine (Baltimore),* 98(33):e16915.

7. Elmore CL, Wu X, Leclerc D, et al (2007). Metabolic derangement of methionine and folate metabolism in mice deficient in methionine synthase reductase. *Mol Genet Metab,* 91(1):85-97.
8. Umay A, Bilgin R, Akgöllü E, Gürkan E, Kis C (2018). Relationship between MTHFR gene polymorphisms (C677T and A1298C) and chronic lymphocytic leukemia in the Turkish population. *Meta Gene,* 17:232-236.
9. Zingg J-M, Jones PA (1997). Genetic and epigenetic aspects of DNA methylation on genome expression, evolution, mutation and carcinogenesis. *Carcinogenesis,* 18(5):869-82.
10. Hussain SR, Naqvi H, Raza ST, et al (2012). Methylenetetrahydrofolate reductase C677T genetic polymorphisms and risk of leukaemia among the North Indian population. *Cancer Epidemiol,* 36(4):e227-31.
11. Reis AAdS, de Alcântara KC, de Farias DLC, et al (2019). The influence of MTHFR C677T polymorphism in chronic lymphocytic leukemia. *Electrophoresis,* 40(12-13):1715-1718 .
12. Abyadeh M, Heydarinejad F, Khakpash M, et al (2020). Association of Apolipoprotein E gene polymorphism with Preeclampsia: a meta-analysis. *Hypertens Pregnancy*, 39(2):196-202.
13. Peterson J, Welch V, Losos M, Tugwell P (2011). The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. *Ottawa: Ottawa Hospital Research Institute.*
14. Wigginton JE, Cutler DJ, Abecasis GR (2005). A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet,* 76(5): 887–893.
15. Huedo-Medina TB, Sánchez-Meca J, Marín-Martínez F, Botella J (2006). Assessing heterogeneity in meta-analysis: Q statistic or I^2 index? *Psychol Methods,* 11(2):193-206.
16. DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials,* 7(3):177-88.
17. Abyadeh M, Djafarian K, Heydarinejad F, et al (2019). Association between Apolipoprotein E Gene Polymorphism and Alzheimer's Disease in an Iranian Population: A Meta-Analysis. *J Mol Neurosci,* 69(4):557-562.
18. Asefi Y, Gohari Mahmoudabad A, Habibian Sezavar A, et al (2020). Association between maternal cadmium exposure and preterm birth: a meta-analysis. *Int J Environ Health Res*:1-10.
19. Egger M, Smith GD, Schneider M, Minder C (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ,* 315(7109): 629–634.
20. Rakonjac N, Ilić V, Šupić G, Cikota B, et al (2008). C677T polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene in patients with chronic lymphocytic leukemia and diffuse large B cell lymphoma. *Serbian Journal of Experimental and Clinical Research,* 9(1):9-12.
21. Gonzalez Ordonez A, FernAandez J, Alvarez CF, et al (2000). Normal frequencies of the C677T genotypes on the methylenetetrahydrofolate reductase (MTHFR) gene among lymphoproliferative disorders but not in multiple myeloma. *Leuk Lymphoma,* 39:607-12.
22. Nückel H, Frey U, Dürig J, Dührsen U, Siffert W (2004). Methylenetetrahydrofolate reductase (MTHFR) gene 677C> T and 1298A> C polymorphisms are associated with differential apoptosis of leukemic B cells in vitro and disease progression in chronic lymphocytic leukemia. *Leukemia,* 18(11):1816-23.
23. Rudd MF, Sellick GS, Allinson R, et al (2004). MTHFR polymorphisms and risk of chronic lymphocytic leukemia. *Cancer Epidemiol Biomarkers Prev,* 13(12):2268-70.
24. Gra OA, Glotov AS, Nikitin EA, et al (2008). Polymorphisms in xenobiotic-metabolizing genes and the risk of chronic lymphocytic leukemia and non-Hodgkin's lymphoma in adult Russian patients. *Am J Hematol,* 83(4):279-87.
25. Gong J-M, Shen Y, Shan W-W, He Y-X (2018). The association between MTHFR polymorphism and cervical cancer. *Sci Rep,* 8:7244.
26. Chen J, Gammon MD, Chan W, et al (2005). One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. *Cancer Res,* 65(4):1606-14.
27. Kennedy DA, Stern SJ, Matok I, et al (2012). Folate intake, MTHFR polymorphisms, and the risk of colorectal cancer: a systematic review and meta-analysis. *J Cancer Epidemiol,* 2012:952508.
28. He L, Shen Y (2017). MTHFR C677T polymorphism and breast, ovarian cancer risk:

a meta-analysis of 19,260 patients and 26,364 controls. *Onco Targets Ther,* 10:227.

29. Kumar P, Rai V (2018). MTHFR C677T polymorphism and risk of esophageal cancer: An updated meta-analysis. *Egyptian Journal of Medical Human Genetics,* 19:273-284.
30. Liu W, Li Y, Li R, et al (2016). Association of MTHFR A1298C polymorphism with breast cancer and/or ovarian cancer risk: an updated meta-analysis. *Afr J Tradit Complement Altern Med,* 13(5):72-86.
31. Tong W, Tong G, Jin D, Lv Q (2018). MTHFR C677T and A1298C polymorphisms and lung cancer risk in a female Chinese population. *Cancer Manag Res,* 10: 4155–4161.