http://pmjournal.ir Original Article

Autumn 2021, Volume 6, Issue 23 (5-9)

Methylenetetrahydrofolate Reductase C677T (rs1801133) Polymorphism and Pemetrexed Treatment Outcome in Patients with Non–Small-Cell Lung Cancer

Mohammad Hadi Abbasian[1], Nafiseh Ansarinejad[2,3], Tayeb Ramim[4], Farshid Fardad[2], Bahareh Abbasi[1*]

[1]Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran
[2]Department of Hematology and Oncology, Hazrat Rasool-e Akram Hospital, Iran University of Medical Sciences, Tehran, Iran
[3]Cancer Pharmacogenetics Research Group (CPGRG), Iran University of Medical Sciences, Tehran, Iran
[4]Department of Health Information Management, School of Health Management and Information Sciences, Iran University of Medical Sciences, Tehran, Iran

**Corresponding author: Bahareh Abbasi, Department of Medical Genetics, National Institute for Genetic Engineering and Biotechnology, Tehran, Iran. Email: Baharehtaleghani@yahoo.com*

DOI: 10.22034/pmj.2021.249035

Submitted: 2021-11-28
Accepted: 2021-12-10

Keywords:
Pemetrexed
NSCLC
Polymorphism
MTHFR
Personalized Medicine

Abstract:

Background: Lung cancer is the first cause of cancer deaths in the world. Pemetrexed is an antifolate drug used as a first or second-line in the treatment of advanced non-small cell lung cancer (NSCLC) patients. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in a folic acid metabolic pathway and a central role in clinical response to pemetrexed. The aim of this study was to investigate the association between rs1801133 polymorphism and the overall survival of metastatic NSCLC patients.
Methods: Thirty-four patients with metastatic lung cancer were treated with pemetrexed-based regimen at Rasoul Akram Hospital, Tehran, Iran. Genomic DNA was extracted from the peripheral blood of patients before initiation of treatment. Genotyping of rs1801133 polymorphism was performed at the National Institute of Genetic Engineering by PCR-RFLP methods. Statistical analysis performed with SPSS software, version 21.0.
Results: Thirty-four patients were enrolled in this study. 21 patients (62%) were male and 13 (38%) were female. The mean age of the patients was 58.90 years. rs1801133 polymorphism were not significantly associated with survival in patients treated with pemetrexed-based chemotherapy.
Conclusion: Previous studies have demonstrated that MTHFR polymorphism may predict survival among pemetrexed-based regimen treated advanced non-squamous NSCLC patients. However, in this study, the examined polymorphisms were not associated with patients' survival.

INTRODUCTION

In 2018, lung cancer, the most common type of cancer, was also the leading cause of cancer-related death worldwide (1). In general, lung cancer is divided into two groups, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Due to the lack of a suitable method for screening and early diagnosis of lung cancer, most patients are diagnosed in the last stages of the disease. Therefore, the 5-year survival of lung cancer patients is less than 15% (2). Pemetrexed is an antifolate drug used as a first or second-line drug for advanced lung cancer chemotherapy (3). Pemetrexed targets several targets in the folate cycle, including thymidylate synthase, dihydrofolate reductase, and glycine amide ribonucleotide formyltransferase. These enzymes serve as biomarkers to predict the effectiveness of pemetrexed treatment (4).

The methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1 and plays a vital role in regulating the level of homocysteine in the body by converting homocysteine to methionine. Genetic polymorphisms are essential in increasing the risk of various diseases, including cancer (5, 6). rs1801133 (C677T) is one of the most clinically important polymorphisms in MTHFR, which converting alanine to valine. This shift leads to increased homocysteine

levels in people who are low in folate (7). Previous studies showed that rs1801133 polymorphism plays a critical role in treating methotrexate and fluorouracil (8). The use of pemetrexed may be associated with severe side effects in some patients (9).

Hematological and gastrointestinal toxicity were reported after pemetrexed therapy in some percentage of patients. These adverse drug reactions lead to dose-reduction and treatment delays that reduce treatment efficacy (10). Identifying pharmacogenetics markers involved in drug metabolism leads to selecting the appropriate drug, increasing the efficiency of treatment, and increasing the overall survival of patients (11). Previous studies showed that race of population influences the results of efficacy chemotherapeutic drugs (12). However, there are no reports on the pharmacogenetics biomarkers of therapeutic effects to pemetrexed-based chemotherapy in the Iranian population.

Therefore, this study investigated the association of rs1801133 polymorphism in the MTHFR gene with response to pemetrexed treatment in metastatic patients with lung cancer. The main question of this study is whether rs1801133 polymorphism in the MTHFR gene is associated with the survival of lung cancer patients treated with a pemetrexed chemotherapy regimen.

Materials and methods

Patients

In this study, 34 patients with metastatic lung cancer were enrolled from April 2019 to March 2020 in Hazrat Rasool-e Akram Hospital, Tehran, Iran. The inclusion criteria for patients was metastatic lung treated with pemetrexed and oxaliplatin who had not previous chemotherapy treatment. Patients' treatment regimens included pemetrexed at a dose of 500 m2 / mg and cisplatin at a 75 m2 / mg dose, which was administered in 4 cycles of 21 days. Patients were followed up for one year after starting treatment. Prior to treatment, informed consent was obtained from all patients to participate in this study. This study was approved by the ethics committee of the National Institute of Genetic Engineering and Biotechnology (IR.NIGEB. EC1398.6.24C)

Genetic analysis

Genomic DNA was isolated from the peripheral blood using the Sinaclone extraction kit (Sinaclon, Iran) PCR reaction in this study was performed in a volume of 25 microliters. Reaction mixture components included 12 µl of deionized water, 10 µl of Mastermix (Amplicon, Denmark), 1 µl of genomic DNA (200 ng / µl) and 1 µl of each primer at a concentration of 1 µM. In this study, primers was designed using NCBI BLAST-Primer and then analyzed by UCSC. The sequence of primers was synthesized by Gene Fanavaran Company (Gene Fanavaran Company, Tehran) PCR reaction steps were performed as follows, initial denaturation step of 5 min at 94 °C followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 67 °C for 45 s and extension at 72 °C for 45 s with a final extension step for 10 min at 72 °C. 5 µl of PCR product digested with 1 µl of HinfI enzyme (Thermo Fisher Scientific, the USA), 25 µl of deionized water and 2 µl of green buffer (Thermo Fisher Scientific, the USA) for 15 minutes at 37 ° C on a hot plate. Restriction fragments were separated by 2 % agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light. Sequences of primers, restriction enzymes, and fragment lengths are shown in Table 1.

Statistical analysis

Data were analyzed by SPSS21 software. T-test was used for comparison of different groups and chi-square was used for qualitative analysis. . Kaplan-Meier method was used for survival analysis.

RESULTS

Of the 34 patients admitted to Hazrat Rasool Hospital, 21 (62%) were male and 13 (38%) were female. The mean age of patients was 58.90 years with a standard deviation of 9.09 years. The minimum age of patients was 40 years and the maximum was 77 years. The frequency of polymorphisms examined in Table 2 and patients' characteristics are listed in Table 3.

Survival of patients after 16 months showed that 28 out of 34 patients (82%) died within the study period. None of the different alleles of rs1801133 polymorphism was significantly associated with patient survival (P> 0.05) (Figure 1).

Table 1. Primer and PCR characteristics

SNP	primer	Restriction enzyme	PCR product length
rs1801133	Forward: gaaggtgcaagatcagag Reverse: ctcaaagaaaagctgcgtgatg	HinfI	CC: 199 and 33 bp TT: 165, 34 and 33 bp. CT : 199,165,34,33

Table 2. Frequency of rs1801133

SNP	Genotype	frequency	%
rs1801133	CC	19	59.9
	CT	11	32.4
	TT	4	11.8

Table 3. Clinical characteristics of patients with non- small-cell lung cancer

variables		Number	Percent
Smoking		18	52
Diabetes		13	38
Kidney diseases		3	8.8
Cardiovascular disease		3	8.8
Metastasis to	Liver	13	38.2
	Brian	24	70

DISCUSSION

Genetic alternation occurs during tumorigenesis, and genetic variants increase cancer susceptibility and heterogeneity of response to cancer treatment (13). Pharmacogenetics of cancer is a promising area to tailoring effective treatment for cancer patients. Our previous study showed that thymidylate synthase ins/del polymorphisms might play a critical role in fluoropyrimidine-based chemotherapy in colorectal and gastric cancer (14).

Previous studies have been recognized pharmacogenetics biomarkers that could predict

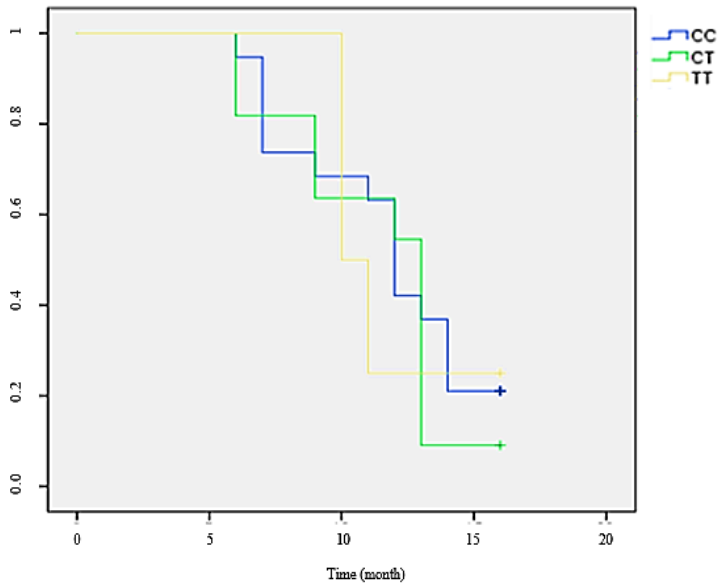

Figure 1. Impact of rs1801133 on overall survival in NSCLC patients treated with pemetrexed

pemetrexed treatment of lung cancer. MTHFR is one of the key regulatory enzymes in folate and homocysteine metabolism and exerts its role by converting 5 and 10 methylenetetrahydrofolates (CH2-THF) to 5-methyltetrahydrofolate (CH3-THF) (15). Two polymorphisms, C677T and A1298C, which significantly alter the enzymatic activity of MTHFR, have been identified in this gene (8). Increasing the level of methyltetrahydrofolate alters the activity of the MTHFR enzyme, which leads to an increase in the activity of thymidylate synthetase (TYMS) and a decrease in the effectiveness of treatment with pemetrexed (16). This study aimed to identify polymorphisms that affect the survival rate of lung cancer. In this study, rs1801133 polymorphism in the MTHFR gene was not associated with overall disease survival.

The association of rs1801133 polymorphism and various cancers has been investigated.

the study by Haerian et al. showed that rs1801133 was not associated with the risk of colorectal cancer in the Iranian population (17). Zhong et al. meta-analysis states that the role of rs1801133 polymorphism in predicting treatment based on fluoropyrimidines in esophageal cancer showed that this polymorphism could not be predicted as a reliable indicator of this type of treatment (18). The meta-analysis of Jiang et al. also showed these results in colorectal cancer (19). Consistent with our result, a study by Vázquez et al. on patients with lung cancer treated with pemetrexed showed that rs1801133 could not predict the therapeutic response to pemetrexed (20). The study by Jung et al found that rs1801133 polymorphism was not associated with the survival of lung cancer patients treated with pemetrexed (21).

In contrast to our study, Krawczyk's study showed that the C / C genotype in the MTHFR gene shortens the recurrence time of patients (22). In the study by Zhang et al., rs1801133 polymorphism has been associated with blood side effects in the 1st and 2nd treatment cycles (23). Lan et al., With CT / TT polymorphism, show a higher prevalence of leukopenia, neutropenia, nausea, and weakness after chemotherapy (24). A study by Hong et al. Showed that rs1801133 polymorphism could be used to predict response to treatment and progression time for lung cancer patients treated with the gemcitabine/platinum regimen (25). Tiseo et al. studied 208 NSCLC patients treated second-line with pemetrexed or pemetrexed plus carboplatin. The result of this study showed that patients with MTHFR-T667T had significantly longer PFS and OS than patients with CC–CT genotypes (26). The meta-analysis of Chen et al. showed that MTHFR rs1801133 polymorphism was significantly associated with susceptibility to lung cancer (27). Another meta-analysis investigated platinum-based chemotherapy (PBC) treatment biomarkers in NSCLC patients and suggested that rs1801133 is reliable biomarker for assessing objective response and progression risk in NSCLC (28).

This is the first study to investigate the therapeutic role of rs1801133 polymorphism in an Iranian lung cancer population. Personalized medicine has revolutionized cancer therapy by predicting adverse drug reactions and tailoring effective medical treatment that maximizes the therapeutic effects of cancer therapy.

However, one of the limitations of the present study is the small sample size. Findings from our study showed that although the survival rate in some genotype groups was higher than other groups, this difference was not statistically significant between them. Finally, it may be concluded that there is no association between MTHFR gene polymorphism and survival after pemetrexed treatment in lung cancer. However, to prove these results, studies with a large sample size are needed.

Conflict of Interest Statement: none

Acknowledgment: This study was funded by the National Institute for Genetic Engineering and Biotechnology, Tehran, Iran.

REFERENCE

1.Abbasian, M. H., Abbasi, B., Ansarinejad, N., Motevalizadeh Ardekani, A., Samizadeh, E., Gohari Moghaddam, K., & Hashemi, M. R. (2018). Association of interleukin-1 gene polymorphism with risk of gastric and colorectal cancers in an Iranian population. Iranian Journal of Immunology, 15(4), 321-328.

2.Abbasian, M. H., Ansarinejad, N., Abbasi, B., Iravani, M., Ramim, T., Hamedi, F., & Ardekani, A. M. (2020). The Role of Dihydropyrimidine Dehydrogenase and Thymidylate Synthase Polymorphisms in Fluoropyrimidine-Based Cancer Chemotherapy in an Iranian Population. Avicenna journal of medical biotechnology, 12(3), 157.

3.Adjei, A. A. (2004). Pemetrexed (ALIMTA), a novel multitargeted antineoplastic agent. Clinical Cancer Research, 10(12), 4276s-4280s.

4.Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68(6), 394-424.

5.Chen, C.-Y., Chang, Y.-L., Shih, J.-Y., Lin, J.-W., Chen, K.-Y., Yang, C.-H., . . . Yang, P.-C. (2011). Thymidylate synthase and dihydrofolate reductase expression in non-small cell lung carcinoma: the association with treatment efficacy of pemetrexed. Lung Cancer, 74(1), 132-138.

6.Chen, F., Wen, T., Lv, Q., & Liu, F. (2020). Associations between Folate Metabolism Enzyme Polymorphisms and Lung Cancer: A Meta-Analysis. Nutrition and Cancer, 72(7), 1211-1218.

7.Corrigan, A., Walker, J., Wickramasinghe, S., Hernandez, M., Newhouse, S., Folarin, A., Marinaki, A. (2014). Pharmacogenetics of pemetrexed combination therapy in lung cancer: pathway analysis reveals novel toxicity associations. The pharmacogenomics journal, 14(5), 411-417.

8.De Mattia, E., & Toffoli, G. (2009). C677T and A1298C MTHFR polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy personalisation. European Journal of Cancer, 45(8), 1333-1351.

9.Haerian, M. S., Haerian, B. S., Molanaei, S., Kosari, F., Sabeti, S., Bidari-Zerehpoosh, F., . . . Zali, M. R. (2016). MTHFR rs1801133 polymorphism and susceptibility to colorectal cancer in Iranian population: evidence of a case–control study and meta-analysis. Pharmacogenomics, 17(17), 1957-1965.
10.Hong, W., Wang, K., Zhang, Y.-p., Kou, J.-y., Hong, D., Su, D., . . . Wang, X.-j. (2013). Methylenetetrahydrofolate reductase C677T polymorphism predicts response and time to progression to gemcitabine-based chemotherapy for advanced non-small cell lung cancer in a Chinese Han population. Journal of Zhejiang University Science B, 14(3), 207-215.
11.Houshmand, M., Seresht-Ahmadi, M., & Alikarami, M. (2020). Evaluation of the relationship between intronic polymorphism TPO gene and risk of type 2 diabetes in Tehran population. Personalized Medicine Journal, 4(16), 9-11.
12.Jiang, H., & Shen, Y. (2019). Methylene tetrahydrofolate reductase (MTHFR) gene rs1801133 C> T polymorphisms and response to 5-FU based chemotherapy in patients with colorectal cancer: a meta-analysis. Pteridines, 30(1), 126-132.
13.Jung, M., Lee, C. H., Park, H. S., Lee, J. H., Kang, Y. A., Kim, S. K., . . . Kim, J. H. (2013). Pharmacogenomic assessment of outcomes of pemetrexed-treated patients with adenocarcinoma of the lung. Yonsei Medical Journal, 54(4), 854-864.
14.Kim, Y.-I. (2009). Role of the MTHFR polymorphisms in cancer risk modification and treatment.
15.Krawczyk, P., Kucharczyk, T., Kowalski, D. M., Powrózek, T., Ramlau, R., Kalinka-Warzocha, E., . . . Kałakucka, K. (2014). Polymorphisms in TS, MTHFR and ERCC1 genes as predictive markers in first-line platinum and pemetrexed therapy in NSCLC patients. Journal of Cancer Research and Clinical Oncology, 140(12), 2047-2057.
16.Kucharczyk, T., Krawczyk, P., Powrózek, T., Kowalski, D. M., Ramlau, R., Kalinka-Warzocha, E., . . . Milanowski, J. (2016). The effectiveness of pemetrexed monotherapy depending on polymorphisms in TS and MTHFR genes as well as clinical factors in advanced NSCLC patients. Pathology & Oncology Research, 22(1), 49-56.
17.Lan, G., Lin, L., Chen, X., Chen, L., & Chen, X. (2017). Correlation between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms and pemetrexed chemotherapy efficacy/toxicity in non-squamous non-small cell lung cancer. Medical science monitor: international medical journal of experimental and clinical research, 23, 5683.
18.Liew, S.-C., & Gupta, E. D. (2015). Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. European Journal of Medical Genetics, 58(1), 1-10.
19.Luo, Y.-H., Luo, L., Wampfler, J. A., Wang, Y., Liu, D., Chen, Y.-M., . . . Yang, P. (2019). 5-year overall survival in patients with lung cancer eligible or ineligible for screening according to US Preventive Services Task Force criteria: a prospective, observational cohort study. The lancet oncology, 20(8), 1098-1108.
20.Malsagova, K. A., Butkova, T. V., Kopylov, A. T., Izotov, A. A., Potoldykova, N. V., Enikeev, D. V., . . . Kaysheva, A. L. (2020). Pharmacogenetic Testing: A Tool for Personalized Drug Therapy Optimization. Pharmaceutics, 12(12), 1240.
21.Nishiyama, A., Katakami, N., Yoshioka, H., Iwasaku, M., Korogi, Y., Hata, A., . . . Uchida, J. (2015). Retrospective efficacy and safety analyses of erlotinib, pemetrexed, and docetaxel in EGFR-mutation-negative patients with previously treated advanced non-squamous non-small-cell lung cancer. Lung Cancer, 89(3), 301-305.
22.Niyikiza, C., Baker, S. D., Seitz, D. E., Walling, J. M., Nelson, K., Rusthoven, J. J., . . . Allen, R. H. (2002). Homocysteine and Methylmalonic Acid: Markers to Predict and Avoid Toxicity from Pemetrexed Therapy 1 Supported by Eli Lilly and Company. 1. Molecular Cancer Therapeutics, 1(7), 545-552.
23.Shen, H., Zhu, M., & Wang, C. (2019). Precision oncology of lung cancer: genetic and genomic differences in Chinese population. NPJ precision oncology, 3(1), 1-8.
24.Tan, L.-M., Qiu, C.-F., Zhu, T., Jin, Y.-X., Li, X., Yin, J.-Y., . . . Liu, Z.-Q. (2017). Genetic polymorphisms and platinum-based chemotherapy treatment outcomes in patients with non-small cell lung cancer: a genetic epidemiology study based meta-analysis. Scientific Reports, 7(1), 1-19.
25.Tiseo, M., Giovannetti, E., Tibaldi, C., Camerini, A., Di Costanzo, F., Barbieri, F., . . . Smit, E. F. (2012). Pharmacogenetic study of patients with advanced non-small cell lung cancer (NSCLC) treated with second-line pemetrexed or pemetrexed–carboplatin. Lung Cancer, 78(1), 92-99.
26.Vázquez, C., Orlova, M., Verzura, M. A., Minatta, J. N., Scibona, P., Jáuregui, E. G., . . . Belloso, W. H. (2019). Severe toxicity in adult patients with lung cancer under treatment with pemetrexed: a prospective cohort study. Journal of Chemotherapy, 31(2), 95-104.
27.Zhang, X., Zhang, D., Huang, L., Li, G., Chen, L., Ma, J., . . . Zhou, C. (2019). Discovery of novel biomarkers of therapeutic responses in Han Chinese pemetrexed-based treated advanced NSCLC patients. Frontiers in Pharmacology, 10, 944.
28.Zhong, L., Fu, Q., Zhou, S., Chen, L., & Peng, Q. (2018). Relevance of MTHFR polymorphisms with response to fluoropyrimidine-based chemotherapy in oesophagogastric cancer: a meta-analysis. BMJ open, 8(5), e020767.