A Genetic Association Study of *MTHFR* C677T Polymorphism with Risk of Metabolic Syndrome: A Systematic Review and Meta-Analysis

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Abstract

Methylenetetrahydrofolate reductase (*MTHFR*) is an enzyme that plays a crucial role as a methyl-group donor in demethylation of homocysteine. The aim of this systematic review and meta-analysis was to study the relationship between *MTHFR* gene polymorphism and metabolic syndrome (MS). We used search engines and databases such as Science Direct, Google Scholar, Embase, Cochrane Library, and PubMed to identify eligible studies up to 2018. The articles were based on keywords including *MTHFR*, mutation, variant, and polymorphism in combination with MS. Data was analyzed using Comprehensive Meta-Analysis version 2.2.064 software. After extracting the data from seven articles, the total number of subjects was 1280 in the patient group and 1374 in the control group. The odds ratio was estimated to be 1.078 for the allele model of T vs. C (95% confidence interval [CI]: 1.626-0.715), 1.157 for the allele model of CC vs. CT (95% CI: 0.829-1.615), 1.020 for the allele model of CT + TT vs. CC (95% CI: 1.611-0.646) and 0.799 for the allele model of TT vs. CC + CT (95% CI: 1.185-0.539). As well, the results showed no statistically significant correlation between polymorphism genotypes of the *MTHFR* gene and MS (P<0.05). In general, this study showed that the presence of C677T polymorphism in the *MTHFR* gene has no effect on the incidence of MS. [GMJ.2019;8:e1472] DOI: [10.31661/gmj.v8i0.1472](https://doi.org/10.31661/gmj.v8i0.1472)

Keywords: *MTHFR*; Metabolic Syndrome; Polymorphism; Variant; Meta-Analysis; Methylenetetrahydrofolate Reductase

Introduction

Methylenetetrahydrofolate reductase (*MTHFR*) is an enzyme that plays an important role as a methyl-group donor in demethylation of homocysteine [1]. Due to the incidence of C677T mutation in the *MTHFR* gene, thymine is replaced by cytosine, followed by the translation of valine instead of alanine in the structure of the produced en...
zyme, resulting in the formation of a temperature-sensitive enzyme and thus reducing its activity [2]. The set of these changes increases the concentration of homocysteine and endothelial dysfunction and accelerates the oxidation of lipoproteins [3]. It has been observed that the levels of homocysteine increase in patients with diabetes mellitus (DM) [4, 5] and hypertension [6, 7]. Some studies also pointed to the relationship between hyperhomocysteinemia and insulin resistance [8-10]. This relationship can be partially justified in light of the correlation of C677T polymorphism (occurring in the MTHFR gene) with hypertension [11], DM [12, 13], and diabetic nephropathy [14]. In recent years, there has been a significant relationship between MTHFR and metabolic syndrome (MS) in certain groups of people with type 2 DM [15] and schizophrenia [16], but these studies have very different results in relation between MTHFR and MS, and no comprehensive study has been done to summarize these outcomes in patients with MS. Regarding the high prevalence of MS and the role of genetic factors in the disease [17], determining the relationship between MTHFR polymorphism and MS can be helpful. In this way, people who are genetically predisposed to the disease can be identified more quickly to fulfill preventive interventions. The aim of this systematic review and meta-analysis was to study the relationship between MTHFR gene polymorphism and MS.

Search Strategies

Search engines and databases including PubMed, Embase, ScienceDirect, Cochrane Library, and Google Scholar were searched to find all English articles up to 2018. The related keywords were extracted using the Medical Subject Heading (MeSH), containing “MTHFR,” “mutation,” “variant,” and “polymorphism” in combination with “MS.” Also, the search was also conducted in the language restriction. Two of the authors reviewed the articles considering the inclusion and exclusion criteria, and the third author reviewed controversies to take the final decision. All articles aiming to investigate the relationship between MTHFR polymorphisms and MS were introduced into the study. Selected arti-

bles for meta-analysis had the following features: evaluation of the relationship between MTHFR gene and MS, number of subjects in case and control groups, access to distribution of genotypes and alleles in case and control groups, considering 95% confidence interval (CI) to estimate odds ratio (OR), original research articles, randomized and controlled articles, and considering gene polymorphism as the main independent variable. Also, when several investigations were conducted on the same population, the latest study was selected to enter into the meta-analysis. The standard information form was used for data collection. Form information included the author name, publication year, study location, genotype type, total number of subjects in case and control groups, genotype distribution in the case and control groups, and the frequency of the dominant allele in both case and control groups. Two of the authors performed the process of extracting information from articles. Hardy-Weinberg equilibrium, genotyping methods qualification, controls source, sample size, and cases representativeness, were examined with a total score of 10 in this scoring. This checklist had been used in previous studies. Besides, scores zero to four were categorized as a weak study, five to seven as an average study, and eight to 10 as a strong article. The allele frequency for gene polymorphism was determined in each study using the allele counting method. OR with 95% CI was used to evaluate the power of correlation between MTHFR gene and MS, followed by the allele model (C vs. T), multiplicative model (CC vs. TT), the dominant model (CC + CT vs. TT), and recessive model (CC vs. CT + TT). Heterogeneity was calculated using measurement test and the random effects model. The I-square (I²) index of 25, 50, and 75 percent showed lower, moderate, and high levels of heterogeneity, respectively. The bias of published articles was examined using a funnel plot versus standard error (SE). Publication bias was evaluated using both Begg’s funnel plot and Egger’s linear regression test. Because of significant heterogeneity of the results (I²>30%), the random effects model, which takes the diversity of the studies into account, was used. All analyses were performed by Comprehensive Meta-Analysis.
version 2.2.064 (CMA) software. A P-value of less than 0.05 was considered as a significance level.

Results

In total, 95 studies were systematically reviewed. Some studies were excluded because of the similarity of the studied samples (n=69), the inappropriateness of the samples and the failure to consider the MS as the main variable (n=22), and inadequate information on alleles (n=4). Finally, seven papers [18-23] were entered into the meta-analysis. Studies characteristics are presented in Table-1. To evaluate the publication bias of studies entered into the meta-analysis, Egger’s test and Begg’s funnel plot were used (Table-2). In all genetic models, the appearance of the shape of the funnel plots was symmetrical (Figure-1). We used Egger’s test to provide the statistical evidence of funnel plot. The findings of the research showed that there is no publication bias in comparison models. Heterogeneities

| Variables | Chedraui et al. | Fakhrzadeh et al. | Kang et al. | Russo et al. | Yang et al. | Zeman et al. |
|-----------|----------------|-------------------|---------|-------------|-------------|--------------|
| Reference | Chen et al.     | [18]              | [19]    | [20]        | [21]        | [15]         | [22]         | [23]         |
| Year      | 2012           | 2008              | 2009    | 2009        | 2002        | 2011         | 2008         |
| County of region | Ecuador | China             | Iran    | Korea       | Italy       | China        | Prague       |
| Study design | PB         | PB                | PB      | HP          | HB          | PB           | PB           |
| Genotyping method | PCR      | PCR               | PCR     | PCR         | PCR         | PCR          | PCR          |
| Cases     | 103            | 118               | 150     | 110         | 50          | 692          | 57           |
| Controls  | 89             | 95                | 76      | 145         | 50          | 878          | 41           |
| CC        | 48             | 34                | 102     | 36          | 36          | 129          | 30           |
| Cases CT  | 45             | 61                | 38      | 60          | 49          | 335          | 19           |
| TT        | 10             | 23                | 10      | 14          | 21          | 228          | 8            |
| CC        | 38             | 57                | 36      | 51          | 31          | 202          | 16           |
| Control CT | 37         | 30                | 31      | 74          | 51          | 431          | 17           |
| TT        | 14             | 8                 | 9       | 20          | 18          | 245          | 18           |
| Minor allele frequency (Cases) | 0.315 | 0.466            | 0.193 | 0.4         | 0.429       | 0.571        | 0.307        |
| Minor allele frequency (Controls) | 0.365 | 0.242            | 0.322 | 0.393       | 0.435       | 0.524        | 0.519        |
| HWE (P-value) | 0.329 | 0.173            | 0.562 | 0.402       | 0.707       | 0.638        | 0.017        |
Table 2. Statistics to Test Publication Bias and Heterogeneity in the Meta-Analysis

| Comparison  | Egger’s regression analysis | Heterogeneity analysis | Model used for the meta-analysis |
|-------------|-----------------------------|------------------------|---------------------------------|
|             | Intercept  | 95% confidence interval | P-value | Q-value | P (Heterogeneity) | I² (%) |                  |
| T vs. C     | -1.05      | -8.21 to 6.10          | 0.670   | 24.27   | <0.001           | 83.521 | Random           |
| TT vs. CC   | -1.26      | -4.52 to 1.99          | 0.362   | 18.66   | 0.005            | 67.852 | Random           |
| CT Vs. CC   | -0.60      | -5.14 to 3.93          | 0.745   | 16.16   | 0.013            | 62.880 | Random           |
| CT+TT vs. CC| -1.79      | -7.98 to 4.39          | 0.489   | 33.68   | <0.001           | 82.190 | Random           |
| TT vs. CT+CC| -2.00      | -3.97 to 2.62          | 0.046   | 15.49   | 0.017            | 61.286 | Random           |

Figure 1. Funnel plot of standard error by log odds ratio
were evaluated by using an $I^2$ test and $Q$-test. Heterogeneity was observed in all the models, i.e., allele (T vs. C), homozygous (TT vs. CC), heterozygous (CT vs. CC), dominant (CT + TT vs. CC), and recessive (TT vs. CT + CC) genotype model, which were included for the meta-analysis. Therefore, the random effects model, which takes the diversity of the studies into account was used for data analysis. Meta-analysis of C677T polymorphism in the MTHFR gene and MS in total consisted of six case-control studies and one cohort study. Available studies in the meta-analysis were used to evaluation of the relationship between MTHFR polymorphism and MS. In general, these studies showed that the total number of subjects was 1280 in the MS patient group and 1374 in the healthy control group. According to the findings of this study (Figure-2), there was no significant relationship between MTHFR 677 C>T polymorphism and MS in the allele model (T vs. C: $P=0.720$; OR = 1.078, 95% CI = 0.715 to 1.626), in the homozygous model (TT vs. CC: $P=0.987$, OR = 1.004, 95% CI = 0.596 to 1.693), in the heterozygous model (CT vs. CC: $P=0.390$, OR = 1.157, 95% CI = 0.829 to 1.615), in the dominant model (CT + TT vs. CC: $P=0.931$, OR = 1.020, 95% CI = 0.646 to 1.611) and in the recessive model (TT vs. CT + CC: $P=0.265$; OR = 0.799, 95% CI = 0.539 to 1.185).

**Discussion**

According to the findings of this study, there was no significant relationship between C677T polymorphism in the MTHFR gene and MS. According to the C677T polymorphism in the MTHFR gene and MS, the result of the present study is in the same line with a study carried out by Russo et al. [15]. They indicate that there is no association between the MTHFR polymorphism and MS in patients with type 2 DM with mild hyperhomocysteinemia [15]. Yamada et al. [24] examined the possible gene responsible for the incidence of MS in 1,788 Japanese individuals, and they found no link between the MTHFR polymorphism and the prevalence of MS. However, Ellingrod et al. observed that the CT mutation in the MTHFR gene predisposes those with schizophrenia taking atypical antipsychotics to MS [25].

This inconsistency is partially justified by epigenetic mechanisms. In this regard, there is a hypothesis that, in addition to inheriting the thrifty gene, epigenetic mechanisms also affect embryonic and postnatal development, and MS underlying disease including insulin resistance, local obesity, dyslipidemia, and hypertension [26]. This generation may even inherit these mechanisms from their fathers or grandparents. Since mitosis occurs during adulthood, epigenetic pathways can affect the expression of the gene in all stages of life. The MTHFR enzyme acts as a methyl-group donor for the remethylation of homocysteine and its conversion into methionine. Methionine consumes methyl group for DNA methylation, especially in CpG pairs. These pairs, which exist in certain regions, act as a promoter for related genes [27]. As a result, environmental and nutritional factors can affect the relationship between MTHFR and MS through these epigenetic mechanisms [28]. It is necessary to carry out comprehensive demographic studies to confirm the conclusion of the present study. According to the results, it is recommended that the necessary interventions should be promoted to change lifestyles to modify the epigenetic mechanisms in society. Flour fortification with folic acid is one of the best available actions. Hypotheses suggest that folic acid fortification can overcome the metabolic block resulting from MTHFR mutation and subsequently affect DNA methylation and gene expression. We had some limitations in this study. These limitations included lack of access to some of the main articles in English and non-English languages.

**Conclusion**

In general, this study showed that the presence of C677T polymorphism in the MTHFR gene has no effect on the incidence of MS. It is suggested to evaluate the effect of folic acid fortification and supplementation on the expression of the MTHFR gene, in particular, those associated with chronic diseases such as hypertension, DM, and MS.

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*Study Name*  
Statistics for each study  
Odds ratio and 95% CI  
Relative weight

| Study Name | Odds ratio | Lower limit | Upper limit | Z Value | p Value | Weight |
|------------|------------|-------------|-------------|---------|---------|--------|
| Chen       | 2.597      | 1.707       | 3.490       | 4.460   | 0.000   | 20.35  |
| Fakhraadeh | 0.531      | 0.286       | 0.989       | 1.996   | 0.046   | 16.25  |
| Kang       | 1.029      | 0.719       | 1.473       | 0.158   | 0.875   | 21.59  |
| Yang       | 1.209      | 1.049       | 1.394       | 2.627   | 0.009   | 25.06  |
| Zeman      | 0.658      | 0.363       | 1.192       | 1.381   | 0.167   | 16.76  |

| Study Name | Odds ratio | Lower limit | Upper limit | Z Value | p Value | Weight |
|------------|------------|-------------|-------------|---------|---------|--------|
| Chen       | 3.409      | 1.937       | 6.270       | 2.947   | 0.003   | 13.00  |
| Fakhraadeh | 0.392      | 0.196       | 0.780       | 1.877   | 0.061   | 12.82  |
| Kang       | 1.000      | 0.545       | 2.121       | 0.020   | 0.984   | 14.00  |
| Russo      | 0.978      | 0.454       | 2.138       | 0.011   | 0.991   | 15.16  |
| Yang       | 1.457      | 1.096       | 1.938       | 2.588   | 0.010   | 21.78  |
| Zeman      | 0.533      | 0.168       | 1.489       | 1.069   | 0.285   | 10.90  |

| Study Name | Odds ratio | Lower limit | Upper limit | Z Value | p Value | Weight |
|------------|------------|-------------|-------------|---------|---------|--------|
| Chen       | 3.706      | 2.092       | 6.566       | 4.488   | 0.000   | 14.16  |
| Fakhraadeh | 0.424      | 0.240       | 0.746       | 2.975   | 0.003   | 14.22  |
| Kang       | 1.115      | 0.660       | 1.983       | 0.498   | 0.619   | 14.74  |
| Russo      | 0.974      | 0.487       | 1.507       | 0.453   | 0.650   | 13.22  |
| Yang       | 1.304      | 1.018       | 1.671       | 2.102   | 0.036   | 21.16  |
| Zeman      | 0.576      | 0.255       | 1.301       | 1.327   | 0.185   | 8.87   |

**Figure 2.** Forest plot analysis for assessing the overall MS risk associated with C677T polymorphism.
in Clinical Research Development Center of Shahid Mohammadi Hospital. The study was approved by the ethics committee of Hormozgan University of Medical Sciences (grant number: 970386 and ethics committee registration code: IR.HUMS.REC.1397.327). We would like to thank all the people who helped us with collecting the data and the searching works.

**Conflict of Interest**

None.

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