Relationship of Methylenetetrahydrofolate Reductase (MTHFR) C677T Variation With Susceptibility of Patients With Ischemic Stroke: A Meta-Analysis

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Abstract

Discovery and validation of genetic factors for multifactorial and polygenic disorders like stroke are needed to make progress in precision medicine. Although some traditional risk factors for stroke have been identified, they do not fully explain the pathophysiological mechanism of ischemic stroke. The research of genetic risk factors is becoming increasingly relevant in the understanding of stroke mechanisms and the finding of population-specific therapeutic targets. The methylenetetrahydrofolate reductase (MTHFR) gene is involved in homocysteine metabolism, and a high homocysteine level is a risk factor for stroke. Using a meta-analysis technique, we investigated the link between the MTHFR C677T gene polymorphism and the risk of ischemic stroke.

We used the electronic databases PubMed, Medline, Embase, and Google Scholar to find articles in the Journal of Stroke. If heterogeneity was more than 50%, pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using a random-effects model; otherwise, a fixed-effects model was used. A total of 67 case-control studies with 17,704 cases and 21,981 controls met our inclusion criteria. The Asian population was represented by 41 studies, whereas the Caucasian population was represented by 26. Under the recessive model, a gene polymorphism at the 677 location of the MTHFR gene is related to an elevated risk of ischemic stroke (OR: 1.29, 95% CI: 1.22–1.37, P < 0.001).

People who have the MTHFR C677T gene polymorphism have a greater risk of stroke than people who do not.

Introduction And Background

Stroke has risen to become the second largest cause of mortality in adults and the third leading cause of disability. Understanding the pathogenesis of stroke necessitates the finding of risk factors [1,2]. Traditional risk factors for ischemic strokes, such as hypertension, diabetes, atrial fibrillation, and smoking, have been extensively researched, although they only account for a minor part of stroke risk [3]. Many previously recognized risk factors for stroke do not fully explain the mechanism of stroke because many stroke victims do not have these risk factors [4]. There was a significant genetic susceptibility to ischemic stroke, according to the evidence from twin and familial aggregation of stroke research. Stroke is a complex disease, according to studies, and it may be caused by shared genetic and environmental variables [5]. It has long been known that a variation in the methylenetetrahydrofolate reductase (MTHFR) gene is linked to the risk of stroke [6].

The 5,10-methylenetetrahydrofolate reductase is an important enzyme that regulates the metabolism of homocysteine (Hcy) levels [7]. MTHFR is an enzyme that helps in the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which further converts Hcy to methionine [8,9]. The MTHFR gene polymorphism is linked to a reduced conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is responsible for the accumulation of Hcy in the bloodstream due to a slowed remethylation reaction from Hcy [10]. Therefore, the alteration in the function of the MTHFR pathway leads to an increased risk of cerebrovascular disease by elevating the level of Hcy in the circulation. Previous epidemiological studies have observed that polymorphism in the MTHFR C677T position is associated with a higher risk of stroke [11,12]. MTHFR gene is considered important to understand the genetic risk of stroke indicated by the published reports. The evidence of precise association can be estimated by conducting a meta-analysis to quantify the pooled effect size based on earlier reported studies in the literature with a similar objective [13]. As a result, we conducted the biggest meta-analysis of
papers published to date to discover the precise relationship between the C677T polymorphism in the MTHFR gene and ischemic stroke.

**Review**

**Methodology and literature search**

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting meta-analysis findings [14]. We conducted a computerized search of MEDLINE, Google Scholar, PubMed, Stroke journal, Web of Science, and Springer for relevant case-control studies from 1997 to 2020. We also looked through references of published manuscripts, editorials, and systematic reviews. The electronic search terms and keywords for obtaining the relevant articles were "MTHFR" OR "MTHFR Polymorphism" OR "MTHFR TT polymorphism" OR "Homocysteine" OR "ischemic stroke in MTHFR TT gene" OR "MTHFR C677T gene in ischemic stroke" OR "MTHFR in stroke." We fixed the filter so that results were limited to humans and articles published in the English language.

**Inclusion and exclusion criteria**

Inclusion criteria included the following: (a) studies that used a case-control study design investigating the relationship between the MTHFR C677T gene and the risk of ischemic stroke; (b) studies including ischemic stroke cases and healthy controls; (c) studies that mentioned the diagnostic criteria for ischemic stroke; (d) studies that reported the genotypic frequencies for both cases and controls; (e) studies with patients aged > 18 years; and (f) studies with enough data for extraction for computing pooled effect size.

Studies were excluded (a) in case genotype frequencies could not be extracted; (b) studies conducted on other subtypes of stroke; (c) cohort studies, cross-sectional studies, and randomized controlled trials; and (d) duplicate publications from the same study with overlapping subjects.

**Extraction of data and evaluation of methodological quality**

We have used the standardized data collection form to extract the data from the included studies. The following important data were extracted for the present study: first author's name, year of article publication, journal in which the article was published, number of genotypes reported in the cases and controls, mean age of cases and controls, and ethnicity. To avoid duplication of the material, we kept only the most recent article or entire study where the same population was reported in multiple publications. Any disputes between the writers were settled through dialogue. For the purposes of the study, ethnicities were divided into two categories: Asian and Caucasian. We also used a quality rating scale created for genetic association studies to assess the methodological quality. Traditional epidemiologic considerations, as well as genetic issues, were included in this scale [15]. The scores ranged from 0 (worst) to 16 (highest).

Pooled odds ratio (OR) with 95% CI was used to determine the pooled effect size [16]. The I² statistic was used to determine statistically significant heterogeneity. We used the random effects model in case of heterogeneity of more than 50%, otherwise, the fixed effect model was used. The probable publication bias was diagnosed using funnel plots and Egger's linear regression test. An ethnicity-based stratified analysis (Asian vs. Caucasian) was carried out. We opted for a two-sided test with <0.05 treated as statistically significant.

**Results**

Previously done meta-analysis studies investigating MTHFR C677T polymorphism and ischemic stroke with OR are shown in Table 1 [12,15-30].
TABLE 1: Pooled ORs of risk from studies investigating methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and ischemic stroke.

| S. No. | Year | Authors | Origin | Sample size, case/control | Total studies | Result (OR, 95% CI) |
|--------|------|---------|--------|--------------------------|---------------|---------------------|
| 1      | 2019 | Chang et al. [12] | China | 0/0 | 9 studies | 1.41 (1.14-1.75) |
| 2      | 2015 | Kumar et al. [15] | India | 6310/8297 | 38 studies | 1.31 (1.19-1.44) |
| 3      | 2014 | Zhang et al. [16] | China | 7990/6941 | 68 studies | 1.86 (1.50-2.31) |
| 4      | 2017 | Abhinand et al. [17] | India | 12,390/16,274 | 72 studies | 1.319 |
| 5      | 2014 | Wu et al. [19] | China | 5207/5383 | 30 studies | 1.62 (1.32-1.99) |
| 6      | 2013 | Yadav et al. [20] | India | 2529/3081 | 26 studies | 2.50 (0.89-6.97) |
| 7      | 2002 | Wald et al. [21] | London | 1217/676 | 7 studies | 1.21 (1.06-1.39) |
| 8      | 2008 | Trabetti [22] | Italy | 4375/4856 | 24 studies | - |
| 9      | 2005 | Cronin et al. [23] | Ireland | 6110/8760 | 32 studies | 1.37 (1.15-1.64) |
| 10     | 2004 | Casas et al. [24] | London | 3387/4597 | 22 studies | 1.24 (1.08-1.42) |
| 11     | 2002 | Clarke et al. [25] | England | 344/300 | 30 studies | - |
| 12     | 2000 | Moller et al. [26] | Denmark | 0/0 | 21 studies | 3.97 |
| 13     | 2008 | Xu et al. [27] | China | 296/216 | 13 studies | 1.55 (1.26-1.90) |
| 14     | 2009 | Xin et al. [28] | China | 2806/7636 | 26 studies | 1.44 (1.14-1.80) |
| 15     | 2016 | Song et al. [29] | China | 4564/6701 | 22 studies | 1.37 (1.16-1.61) |
| 16     | 2013 | Li et al. [30] | China | 2223/2936 | 19 studies | 1.28 (1.17-1.40) |

A total of 67 studies that met the inclusion criteria were included in this study, having 17,704 cases and 21,981 controls. The studies were conducted from the period of 1997 to 2020. There were 41 studies from the Asian population and 26 from the Caucasian population. Figure 1 shows the search results. The characteristics of the included studies are presented in Table 2. In this meta-analysis, all studies’ genotype data were following the Hardy-Weinberg equilibrium. All included studies’ methodological quality scores ranged from 3.5 to a maximum of 14 (Table 2). MTHFR gene polymorphism at 677 locations is significantly associated with the increased risk of ischemic stroke (OR: 1.29, 95% CI: 1.22-1.37, P < 0.001) (Figure 2). Meta-regression analysis has shown no significant influence on mean age (P = 0.693) (Figure 3), ethnicity (P = 0.71) (Figure 4), and methodological quality in the study population (P = 0.977) with effect size (Figure 5). We stratified the data into two groups based on the results of studies conducted on Asian and Caucasian populations. Subgroup analysis (year-wise) has shown no association in the studies having an OR and corresponding 95% CIs of 1.30 (1.22-1.39) for the Asian population and 1.23 (1.08-1.40) for the Caucasian population (Figure 6).
**FIGURE 1: PRISMA flow diagram.**

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

| S. No. | Year | Study            | Origin | Sample size, case/control | Hardy-Weinberg equilibrium (HWE) | Total, male/female | Age | Quality score |
|--------|------|------------------|--------|---------------------------|----------------------------------|-------------------|-----|--------------|
| 1      | 1997 | Markus et al. [31] | London | 345/161                    | Yes                              | 287/0             | 66.4| 12           |
| 2      | 1998 | Morita et al. [32] | Japan  | 256/325                    | Yes                              | 0                 | 51  | 11           |
| 3      | 1998 | Pepe et al. [33]  | Italia | 72/198                     | No                               | 72/198            | 41.4| 7            |
| 4      | 1998 | Salooja et al. [34] | London | 242/173                    | No                               | 68/69             | 68  | 10           |
| 5      | 1998 | Kostulas et al. [35] | Sweden | 126/126                    | Yes                              | 0                 | 0   | 9            |
| 6      | 1999 | Press et al. [36] | Portland | 167/115                  | Yes                              | 126/52            | 66  | 6            |
| 7      | 1999 | Lalouschek et al. [37] | Austria | 96/96                      | No                               | 58/38             | 0   | 7            |
| 8      | 1999 | Hamon et al. [38] | Ireland | 174/183                   | No                               | 183/174           | 75.9| 8            |
| 9      | 2000 | Eikelboom et al. [39] | Australia | 219/205               | Yes                              | 195/219           | 66.6| 12           |
| 10     | 2000 | Voetsch et al. [40] | Brazil | 153/225                    | Yes                              | 153/225           | 0   | 9            |
| 11     | 2000 | Zheng et al. [41] | China  | 115/122                    | Yes                              | 18/12             | 48  | 9            |
| Year | Authors | Country | Patients | Control | Yes No | Yes % | Control % |
|------|---------|---------|----------|---------|--------|--------|-----------|
| 2001 | Topić et al. | Croatia | 56/124 | No | 92/0 | 64.0 | 3.5 |
| 2001 | Zhang et al. | China | 102/100 | Yes | 102/100 | 57.5 | 7.5 |
| 2001 | Wu et al. | Japan | 77/229 | Yes | 77/229 | 60.5 | 10 |
| 2001 | Lopaciuk et al. | Poland | 100/238 | No | 51/49 | 38.1 | 10 |
| 2002 | Yingdong et al. | China | 43/42 | Yes | 0 | 0 | 7 |
| 2002 | Huang et al. | China | 49/50 | Yes | 0 | 55 | 8 |
| 2002 | Grossmann et al. | Germany | 93/186 | No | 140/139 | 0 | 9 |
| 2002 | Madonna et al. | Italy | 132/262 | No | 117/145 | 37.2 | 10 |
| 2002 | Mcllroy et al. | Ireland | 63/71 | No | 71 | 74.1 | 4.5 |
| 2003 | Szolnoki et al. | Hungary | 867/743 | Yes | 853/757 | 60.8 | 14 |
| 2003 | Li et al. | China | 1320/1832 | Yes | 195/198 | 61.1 | 11 |
| 2003 | Yeh et al. | China | 213/200 | No | 17/167 | 45.1 | 7 |
| 2004 | Wu et al. | China | 132/262 | No | 117/145 | 37.2 | 10 |
| 2004 | McIlroy et al. | Ireland | 63/71 | No | 71 | 74.1 | 4.5 |
| 2004 | Sazci et al. | Turkey | 867/743 | Yes | 853/757 | 60.8 | 14 |
| 2004 | Pezzini et al. | Italy | 1320/1832 | No | 195/198 | 61.1 | 11 |
| 2005 | Alluri et al. | India | 69/49 | No | 30/10 | 0 | 10 |
| 2005 | Kawamoto et al. | Japan | 97/241 | Yes | 17/167 | 45.1 | 7 |
| 2005 | Pezzini et al. | Italy | 132/262 | No | 117/145 | 37.2 | 10 |
| 2005 | Sazci et al. | Turkey | 867/743 | Yes | 853/757 | 60.8 | 14 |
| 2005 | Gao et al. | China | 1320/1832 | Yes | 195/198 | 61.1 | 11 |
| 2006 | Hermans et al. | Belgium | 23/142 | Yes | 23/154 | 69.4 | 7 |
| 2006 | Panigrahi et al. | India | 32/60 | No | 0 | 25 | 7 |
| 2006 | Dikmen et al. | Turkey | 203/55 | Yes | 126/132 | 61.1 | 9 |
| 2006 | Shinjo et al. | Brazil | 127/126 | Yes | 125/0 | 63.8 | 7 |
| 2006 | Zhang et al. | China | 245/282 | Yes | 255/282 | 0 | 8 |
| 2008 | Shi et al. | China | 97/99 | No | 159/37 | 38.7 | 11 |
| 2008 | Moe et al. | Singapore | 120/207 | Yes | 233/94 | 60.8 | 10 |
| 2009 | Biswas et al. | India | 120/120 | Yes | 0 | 0 | 8 |
| 2009 | Al-Allawi et al. | Iraq | 70/50 | No | 64/56 | 0 | 12 |
| 2009 | Sabino et al. | Brazil | 21/37 | No | 24/34 | 60.8 | 8 |
| 2010 | Han et al. | Korea | 263/234 | Yes | 267/234 | 60.9 | 9 |
| 2010 | Salem-Berrabah et al. | Tunisia | 50/97 | No | 53/97 | 44.2 | 11.5 |
| 2010 | Isordia-Salas et al. | Mexico | 178/183 | Yes | 122/120 | 39.4 | 10 |
| 2011 | Mohamed et al. | Malaysia | 72/72 | Yes | 163/129 | 60.8 | 9 |
|   | Year | Study                          | Country     | Sample Size | Control Group | N/A      | Odds Ratio | 95% CI    | p-Value |
|---|------|--------------------------------|-------------|-------------|---------------|----------|------------|-----------|---------|
| 49 | 2011 | They-They et al. [79]         | Morocco     | 91/182      | Yes           | 91/182   | 47.5       | 10        |
| 50 | 2011 | Somarajan et al. [80]         | India       | 207/188     | Yes           | 0        | 0          | 11        |
| 51 | 2011 | Arsene et al. [81]            | Romania     | 67/60       | No            | 53/97    | 70         | 9         |
| 52 | 2011 | Mohamed et al. [78]           | Malaysia    | 150/142     | Yes           | 163/129  | 60.8       | 9         |
| 53 | 2012 | Xiong et al. [82]             | China       | 89/102      | Yes           | 0/53     | 68.1       | 9         |
| 54 | 2012 | Alfan et al. [83]             | China       | 512/500     | No            | 310/202  | 58.4       | 8         |
| 55 | 2013 | Fekih-Mrissa et al. [84]      | Tunisia     | 84/100      | No            | 121/63   | 53         | 10        |
| 56 | 2014 | Zhou et al. [85]              | China       | 543/655     | No            | 748/452  | 66         | 8         |
| 57 | 2015 | Al-Gazally et al. [86]        | Iran        | 30/30       | No            | 90/110   | 57.3       | 6         |
| 58 | 2015 | Nissar et al. [87]            | India       | 70/160      | Yes           | 133/97   | 43.5       | 1         |
| 59 | 2015 | Kumar et al. [15]             | India       | 6310/8297   | Yes           | 0        | 0          | 10        |
| 60 | 2015 | Das et al. [88]               | India       | 620/620     | Yes           | 862/388  | 50         | 11        |
| 61 | 2015 | Lv et al. [89]                | China       | 199/241     | Yes           | 245/195  | 68         | 11        |
| 62 | 2016 | Kumar et al. [90]             | India       | 250/250     | Yes           | 406/97   | 51.9       | 11        |
| 63 | 2017 | Ma et al. [91]                | China       | 236/390     | Yes           | 368/258  | 64         | 13        |
| 64 | 2017 | Li et al. [92]                | China       | 300/261     | No            | 257/304  | 64         | 12        |
| 65 | 2018 | Hou et al. [93]               | China       | 1967/2565   | Yes           | 2858/0   | 66.9       | 12        |
| 66 | 2019 | Hashemi et al. [94]           | Southeast Iran | 106/157     | No            | 111/154  | 37.1       | 9.5       |
| 67 | 2021 | Mazdeh et al. [95]            | Iran        | 318/400     | Yes           | 318/400  | 0          | 14        |

**TABLE 2: Characteristics of studies included in the meta-analysis on the association between MTHFR C677T polymorphism and ischemic stroke.**

MTHFR: methylenetetrahydrofolate reductase.
FIGURE 2: Forest plot and pooled ORs of risk from studies investigating methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and ischemic stroke.

Reference citations: [83, 73, 86, 80, 81, 85, 72, 53, 88, 67, 39, 84, 48, 75, 38, 84, 85, 93, 47, 77, 61, 35, 51, 95, 70, 75, 92, 45, 86, 80, 91, 49, 31, 95, 86, 71, 70, 87, 66, 33, 50, 62, 63, 74, 76, 34, 63, 70, 68, 80, 51, 79, 42, 56, 40, 44, 55, 82, 54, 46, 43, 69, 41, 85].
FIGURE 3: Meta-regression analysis to determine the influence of mean age in the study population with effect size.

FIGURE 4: Meta-regression analysis to determine the influence of ethnicity in the study population with effect size.
FIGURE 5: Meta-regression analysis to determine the influence of methodological quality in the study population with effect size.
Publication bias

The probabilities of publication bias arising from the published literature were examined using a funnel plot and the Beggs’s and Egger’s tests. We observed that there was significant publication bias ($P < 0.001$), indicating that there were probabilities of publication bias (Figure 7).
Discussion

Our meta-analysis, which included 67 studies, observed that variation at the C677T position of the MTHFR gene might be associated with an increased risk to develop ischemic stroke.

Earlier meta-analyses [16,17] with a substantial number of studies have also shown the significant relationship between C677T variation of the MTHFR gene and increased risk of ischemic stroke (Table 1). However, earlier meta-analyses had limitations to obtain the precise estimate of risk associated with MTHFR gene polymorphism for the risk of ischemic stroke. The meta-analysis published by Zhang et al. [16] recruited studies (68 studies) only from the Chinese population, which limits the generalizability of the study findings. Another meta-analysis reported by Abhinand et al. [17] in 2017 had limitations with the inclusion of the same study multiple times, and inadequate statistical analysis to draw a precise conclusion. This meta-analysis also included studies with cervical artery dissections and venous thrombosis, which would have influenced the pooled effect size to derive a homogenous effect size.

In view of these, our meta-analysis is the largest meta-analysis that used the robust statistical method and methodological quality to derive the precise conclusion regarding the relationship of MTHFR gene polymorphism at 677 positions with the risk of ischemic stroke. In the stratified analysis, the association was found to be higher in the Asian population (OR: 1.30, 95% CI: 1.22-1.39) as compared to the Caucasian population (OR: 1.23, 95% CI: 1.08-1.40). However, in meta-regression analysis, ethnicity did not contribute to the significant heterogeneity in the pooled effect size. These findings indicate that similar type of association between MTHFR gene polymorphism and the risk of ischemic stroke in both Asian and Caucasian populations. Our meta-regression analysis to explore the source of variation in effect size did not observe the significant influence of mean age, methodological quality, and year of publication of articles on the pooled effect size. These observations further strengthen the homogeneous effect of the MTHFR gene polymorphism with an increased risk of ischemic stroke.

MTHFR polymorphism leads to a higher level of Hcy. Hcy is a sulfur-containing amino acid and its remethylation leads to the formation of methionine. In the remethylation process of methionine, the methyl donor for the conversion of Hcy to methionine is done by the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate by the enzyme MTHFR. Elevated plasma Hcy levels can occur due to defective remethylation of Hcy to methionine because mutations in the MTHFR gene could lead to decreased activity of the MTHFR enzyme [16-18]. Stroke guidelines have included the examination of the Hcy biomarker in young stroke patients as a higher level of Hcy was found to be associated with an increased risk of stroke. It could be effectively treated with vitamin B12 and folic acid supplementation. It has been observed that vitamin supplementation effectively controls the level of Hcy and thereby reduces the risk of stroke [8]. The findings of the present study further strengthen the routine examination of MTHFR gene polymorphism for the prevention of stroke along with Hcy levels.
Conclusions

This meta-analysis sustains the notion of the association of MTHFR gene polymorphism with an increased risk of ischemic stroke. The observed pooled effect size had insignificant heterogeneity, which further strengthens the findings observed in the current study. The study is limited by the presence of publication bias. The association of MTHFR gene polymorphism was found to be higher in the Asian population compared to Caucasians. MTHFR gene polymorphism screening may be included in the guidelines for the prevention and screening of subjects with higher susceptibility to stroke.

Additional Information

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Acknowledgements

Dr. Pramod Kumar and Aparna Mishra contributed equally to the work and should be considered co-first authors. Notes on contributors: PK and AM have extracted the data and written the manuscript. All drafting and editing are done by MKP. Data analysis is done by VV. Final approval, conceptualization, and statistics are done by AK.

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