Association of MTHFR C677T and A1298C Polymorphisms with Glaucoma Risk: a Systematic Review Meta-Analysis based 42 Case-Control Studies

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Accepted: February 14th, 2019

Abstract

Aim: Several epidemiological studies have been performed to explore the association of MTHFR polymorphisms with glaucoma risk. However, the results were inconsistent or even inconclusive. Hence, we performed a meta-analysis to evaluate the association of MTHFR C677T and A1298C polymorphisms with glaucoma risk.

Methods: A comprehensive literature search on PubMed, Google Scholar, EMBASE, and CNKI databases was performed to find all eligible studies up to January 30, 2019. The pooled odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of such association.

Results: A total of 42 case-control studies including 33 studies for MTHFR C677T and nine studies for A1298C polymorphism were selected. Pooled results showed that there was no significant association between the MTHFR C677T polymorphism and glaucoma risk. Similarly, no associations were found in subgroup analysis based on ethnicity and glaucoma type. However, there was a significant association between the A1298C polymorphism and the increased risk of glaucoma under heterozygote model (OR=0.765, 95% CI=0.626-0.935, P=0.009). Moreover, the significant association between MTHFR A1298C polymorphism and glaucoma were found by ethnicity and primary open angle glaucoma (POAG).

Conclusions: The present meta-analysis revealed that MTHFR A1298C polymorphism is significantly associated with the increased risk of glaucoma, but not MTHFR C677T polymorphism.

Keywords: glaucoma, methylenetetrahydrofolate reductase, polymorphism, meta-analysis

Introduction

Glaucoma is an optic neuropathy in which the optic nerve is damaged with typical loss of nerve fibers and increasing cupping of the optic disc, leading to progressive, irreversible loss of vision [1,2]. A leading cause of all blindness worldwide, secondary to cataracts, glaucoma is the main cause of irreversible vision loss [3]. It is estimated that more than 60 million people had
glaucoma in 2010, 8.4 million of whom are bilaterally blind as a result of this disease [4]. In general, glaucoma might be classified in three major categories: primary open angle glaucoma (POAG), primary congenital glaucoma (PCG) and primary angle-closure glaucoma (PACG) [5].

Glaucoma is a multifactorial disease involving both environmental and genetic factors [6,7]. During the past decade, molecular genetic studies of glaucoma have yielded some success. The importance of genetic factors in the etiology of glaucoma is supported by genome-wide association studies (GWASs) [8]. Recently, several candidate novel loci have been identified in a GWAS for POAG (e.g., ABCA1, AFAP1, GMDS, PMM2, TGFBR3, FNDC3B, ARHGEF12, GAS7, FOXC1, ATXN2, TXNRD2); PACG (e.g., EPDR1, CHAT, GLIS3, FERMT2, DPM2-FAM102); and exfoliation syndrome (XFS) glaucoma (CACNA1A) [8,9]. Furthermore, several epidemiological studies have reported a link between methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and glaucoma [10,11].

The MTHFR gene is located on chromosome 1p36.3 [12,13]. It is an important regulatory enzyme in the folate related one carbon metabolism, which is responsible for catalyzing 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate [14,15]. In addition, MTHFR plays an important role by directing folate metabolites through the DNA methylation pathways [12]. An increased level of plasma homocysteine (Hcy) has been observed in patients with glaucoma [16]. The MTHFR gene is encoded by 11 exons and includes several SNP, some of which have functional relevance and result in high Hcy level. Many studies have shown an increased risk of glaucoma in patients with MTHFR C677T and A1298C polymorphism. However, results from these studies were inconsistent or inconclusive. It was suggested that this inconsistency might be related to the single studies with low statistical power, publication biases, and ethnicity differences. Thus, we have performed the current systematic review and meta-analysis to collecting and summarizing the evidence on the association of MTHFR C677T and A1298C polymorphisms with the risk of glaucoma.

Materials and Methods

Study Identification and Selection

We have performed a comprehensive literature search using PubMed, Web of Science, Google Scholar, Cochrane Library, Embase, and Chinese Biomedical Literature database (CBM) databases to identify studies that evaluated the association between MTHFR C677T polymorphism and the risk of glaucoma up to October 2018, with the following keywords: “Methylenetetrahydrofolate reductase”, “MTHFR”, “MTHFR C677T”, or “MTHFR A1298C” and “polymorphism”, “mutation”, or “variant” and “glaucoma” and “primary open-angle glaucoma” or “POAG” and “pseudoexfoliation glaucoma” or “PXG”, “pseudoexfoliation syndrome with glaucoma” or “PEXG” and “normal-tension glaucoma” or “NTG” and “primary angle-closure glaucoma” or “PACG” and “juvenile-onset open-angle glaucoma” or “JOAG”. We have retrieved any article matching the keywords and we evaluated it by reading the title and abstract. In addition, we have screened the references lists of the retrieved articles for original papers.

Inclusion and Exclusion Criteria

The following criteria were used for the study selection: 1) a case-control study evaluating the association of MTHFR C677T and A1298C polymorphisms with the risk of glaucoma and its types; 2) case-control or cohort studies; 3) sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI); 4) no overlapping data. In addition, if studies had the same or overlapping data, we have included only the largest study in the final analysis. The major excluding criteria for studies were the following: (1) not glaucoma research, (2) reviews, letters or case reports, (3) duplicate of previous publication, and (4) and those articles without definite information of genotypes.

Data Extraction

We have extracted information carefully from all the eligible studies independently by two investigators based on the above listed inclusion criteria. The following data were collected from each study: the first author’s
name, the year of publication, ethnicity, country of origin, glaucoma type, genotyping method, source of control groups (population-based or hospital-based controls), total number of cases and controls, the frequencies of genotypes, minor allele frequencies (MAFs), and Hardy-Weinberg equilibrium (HWE) test in control subjects. Allele frequencies were calculated from the corresponding genotype distributions using an online website. Finally, the extracted data in terms of accuracy and any discrepancy between these two authors was resolved by reaching a consensus through discussion or the involvement of a third author who made the final decision through discussions.

Statistical Analysis

Pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to assess the association of MTHFR C677T and A1298C polymorphisms with the risk of glaucoma. The significance of the pooled OR was determined by the Z-test. The pooled ORs were performed under five genetic models, i.e., allele (B vs. A), homozygote (BB vs. AA), heterozygote (BA vs. AA), dominant (BB+BA vs. AA), and recessive (BB vs. BA+AA), which a “A” denotes a major allele; “B” denotes a minor allele. Heterogeneity (between-study inconsistency) was assessed by the Cochran X²-based Q test (Heterogeneity was considered statistically significant if P<0.10) and the I² statistics. An I² value of 0% represents no heterogeneity, with values of 25%, 50%, 75%, or more represent low, moderate, high, and extreme heterogeneity, respectively. A fixed effect model (Mantel-Haenszel method) was used to calculate pooled OR when there was no heterogeneity among the studies. Otherwise, the fixed-effects model (Mantel-Haenszel approach) was used. We have calculated the Hardy-Weinberg equilbriums (HWEs) with goodness-of-fit tests (i.e., chi-square or Fisher’s exact tests). In addition, one-way sensitivity analyses were carried out by consecutively omitting one study at a time to assess power of the meta-analysis [15]. In addition, sensitivity analysis was also performed, excluding studies whose allele frequencies in controls exhibited a significant deviation from the Hardy-Weinberg equilibrium (HWE), given that the deviation may denote bias. Deviation of HWE may reflect methodological problems such as genotyping errors, population stratification or selection bias. Visual inspection of the asymmetry of funnel plots was carried out to assess potential publication bias. Begg’s funnel plot, a scatter plot of effect against a measure of study size was used as a visual aid to detect bias or systematic heterogeneity. Publication bias was assessed by Egger’s test (p<0.05 was considered statistically significant). If publication bias existed, the Duval and Tweedie non-parametric “trim and fill” method was used to adjust for it. A meta-regression analysis was carried out to identify the major sources of between-studies variation in the results, using the log of the ORs from each study as dependent variables, and ethnicity and source of controls as the possible sources of heterogeneity. All the statistical calculations were performed using Comprehensive Meta-Analysis (CMA) software version 2.0 (Biostat, USA). Two-sided P-values < 0.05 were considered statistically significant.

Results

Study Selection and Characteristics

A flow diagram schematizing the inclusion and exclusion process of identified articles with the inclusion criteria is presented in Fig. 1.
After a comprehensive search, a total of 342 articles were identified. Of these studies, the first screening excluded 216 as duplicates or not relevant, leaving 126 for further selection. Among the remaining studies, 84 articles were excluded because they were review articles, letters to editors, previous meta-analyses, not relevant to MTHFR C677T and A1298C, not case-control studies, evaluated other diseases instead of glaucoma, case reports, and other polymorphisms of MTHFR gene. Finally, a total of 42 case-control studies including 33 studies (in 19 publications) with 3,504 cases and 2,525 controls for MTHFR C677T [9–11,17–31] and nine studies (in six publications) with 1,073 cases and 775 controls for A1298C [11,19–21,23,29] were selected. The main characteristics of studies included in the current meta-analysis are presented in Tables 1 and 2.

Table 1. Characteristics of the studies included in the MTHFR C677T polymorphism meta-analysis

| First author | Country (Ethnicity) | Type | Case | Control | SOC | Genotyping Technique | Genotypes | Cases | Alleles | Control |
|--------------|---------------------|------|------|---------|-----|---------------------|------------|------|---------|---------|
| Bleich 2002   | Germany (Caucasian) | POAG | 18   | 19      | PB  | RT-PCR              | CC         | 5    | 11      | 2       | 21 15 |
| Linnemann 2005 | Germany (Caucasian) | POAG | 76   | 71      | HB  | RT-PCR              | CC         | 32   | 37      | 7       | 101 51 |
| Moschos 2006 | Greece (Caucasian) | POAG | 204  | 211     | HB  | PCR-RFLP            | TT         | 139  | 71      | 14      | 309 99 |
| Matsuoka 2006 | Japan (Asian)       | POAG | 138  | 14      | HB  | PCR-RFLP            | TT         | 72   | 50      | 16      | 194 82 |
| Malhotra 2008 | Pakistan (Asian)   | NTG  | 86   | 106     | HB  | Sequencing          | TT         | 48   | 8       | 4       | 194 16 |
| Finger 2006   | USA (Caucasian)     | POAG | 178  | 168     | PB  | PCR-RFLP            | TT         | 41   | 9       | 2       | 194 27 |
| Zetterberg 2007 | Romania (Caucasian) | POAG | 145  | 41      | PB  | PCR-RFLP            | TT         | 12   | 9       | 4       | 53 37 |
| Fan 2008      | China (Asian)       | NTG  | 61   | 50      | HB  | TaqMan               | CC         | 20   | 31      | 7       | 78 44 |
| Michael 2008  | Pakistan (Asian)   | PCR  | 90   | 70      | HB  | PCR-RFLP            | TT         | 70   | 20      | 0       | 160 20 |
| Michael 2009  | Pakistan (Asian)   | PCR  | 173  | 143     | HB  | PCR-RFLP            | TT         | 123  | 49      | 1       | 295 51 |
| Clement 2009  | Australia (Caucasian) | PCR | 48   | 42      | PB  | RT-PCR               | TT         | 18   | 23      | 7       | 59 37 |
| Woo 2009      | Korea (Asian)       | NTG  | 78   | 100     | PB  | PCR-RFLP            | TT         | 25   | 34      | 19      | 84 72 |
| Fan 2010      | Hong Kong (China)  | NTG  | 100  | 201     | PB  | Sequencing           | TT         | 11   | 71      | 154     | 110 400 |
| Nifvekhan 2012 | Iran (Asian)       | POAG | 50   | 50      | CC  | TaqMan               | TT         | 0    | 20      | 26      | 22 78 |
| Shi 2013      | China (Asian)       | NTG  | 231  | 306     | HB  | TaqMan               | TT         | 46   | 31      | 8       | 123 47 |
| Zacherlei 2014 | Greece (Caucasian) | PCR  | 144  | 173     | HB  | PCR-RFLP            | TT         | 101  | 35      | 8       | 237 51 |
| Al-Shahrami 2015 | Saudi Arabia (Asian) | PCR | 144  | 173     | HB  | PCR-RFLP            | TT         | 73   | 14      | 0       | 160 14 |
| Dunn 2015     | India (Indian)      | NTG  | 30   | 128     | CC  | TaqMan               | TT         | 24   | 31      | 11      | 75 53 |

POAG = primary open angle glaucoma, PXFG = pseudoexfoliation glaucoma, PEXG = pseudoexfoliation syndrome with glaucoma, NTG = normal-tension glaucoma, PAGG = primary angle-closure glaucoma, HTG = high-tension glaucoma, JAG = juvenile-onset open-angle glaucoma, SOC = source of control, PCR-RFLP = Polymerase chain reaction-restriction fragment length polymorphism, RT-PCR = Real-time polymerase chain reaction, NR = Not report, PB = Population-based, HB = Hospital-based, MAFs = Minor Allele Frequency, HWE = Hardy–Weinberg equilibrium in control population

Table 2. Characteristics of the studies included in the MTHFR A1298C polymorphism meta-analysis

| First author | Country (Ethnicity) | Type | Case | Control | SOC | Genotyping Technique | Genotypes | Cases | Alleles | Control |
|--------------|---------------------|------|------|---------|-----|---------------------|------------|------|---------|---------|
| Malhotra 2006 | Japan (Asian)       | NTG  | 133  | 106     | HB  | Sequencing          | TT         | 139  | 71      | 14      | 309 99 |
| Zetterberg 2007 | Romania (Caucasian) | NTG  | 133  | 106     | HB  | Sequencing          | TT         | 119  | 97      | 27      | 335 151 |
| Fan 2008      | USA (Caucasian)     | PCR  | 57   | 50      | HB  | TaqMan               | TT         | 26   | 20      | 11      | 72 22 |
| Michael 2009  | Pakistan (Asian)   | PCR  | 122  | 146     | HB  | PCR-RFLP            | TT         | 34   | 76      | 12      | 144 100 |
| Woo 2009      | Korea (Asian)       | NTG  | 78   | 156     | HB  | PCR-RFLP            | TT         | 57   | 19      | 2       | 133 23 |
| Zacharaki 2014 | Greece (Caucasian) | PCR  | 64   | 130     | HB  | TaqMan               | TT         | 13   | 31      | 22      | 53 75 |

POAG = primary open angle glaucoma, PXFG = pseudoexfoliation glaucoma, NTG = normal-tension glaucoma, PAGG = primary angle-closure glaucoma, SOC = source of control, PCR-RFLP = Polymerase chain reaction-restriction fragment length polymorphism, HB = Hospital-based, MAFs = Minor Allele Frequency, HWE = Hardy–Weinberg equilibrium in control population
Among these studies, six types of glaucoma, including primary open angle glaucoma (POAG), pseudoexfoliation glaucoma (PXFG), or pseudoexfoliation syndrome with glaucoma (PEXG), normal-tension glaucoma (NTG), primary angle-closure glaucoma (PACG), high-tension glaucoma (HTG), and juvenile-onset open-angle glaucoma (JOAG) were involved. Among the selected studies, 23 case-control studies were conducted in the Asians, 18 studies were conducted in the Caucasians, and one study was conducted in the Latinos. Genotyping methods used in the studies include PCR-RFLP, Real-time PCR, TaqMan, and sequencing. The genotype frequencies in the control group for three publications did not fit well in the Hardy-Weinberg equilibrium (P>0.05).

Table 3. Summary risk estimates for association between MTHFR C677T polymorphism and glaucoma risk

| Subgroup | Type of Model | Genotype Model | Heterogeneity | Odds ratio | Publication Bias |
|----------|---------------|----------------|---------------|------------|-----------------|
| Overall  | Random        | T vs. C        | F (%) ≤0.001  | OR 95% CI  | Z OR     |
|          |               | TT vs. CC      | 56.96         | 1.120      | 0.994-1.262 |
|          |               | TC vs. CC      | 26.28         | 0.905      | 0.899-1.299 |
|          |               | TT+TC vs. CC   | 34.76         | 0.027      | 0.899-1.188 |
|          |               | TT vs. CC+CC   | 55.75         | ≤0.001     | 0.948-1.306 |
| By Glaucoma Type |                |                |               |            |                |
| POAG     | Fixed         | T vs. C        | 66.56         | ≤0.001     | 1.199      |
|          |               | TT vs. CC      | 36.99         | 0.087      | 1.120      |
|          |               | TC vs. CC      | 59.03         | 0.032      | 1.127      |
|          |               | TT+TC vs. CC   | 64.72         | ≤0.001     | 1.149      |
|          |               | TT vs. CC+CC   | 11.19         | 0.332      | 1.124      |
| PACG     | Fixed         | T vs. C        | 29.71         | 0.223      | 0.99       |
|          |               | TT vs. CC      | 69.18         | 0.021      | 2.356      |
|          |               | TC vs. CC      | 0.00          | 0.946      | 0.820      |
|          |               | TT+TC vs. CC   | 0.00          | 0.807      | 0.903      |
|          |               | TT vs. CC+CC   | 68.98         | 0.022      | 2.594      |
| PXFG + PEXG | Fixed         | T vs. C        | 39.66         | 0.127      | 1.151      |
|          |               | TT vs. CC      | 9.68          | 0.355      | 1.271      |
|          |               | TC vs. CC      | 45.22         | 0.090      | 1.101      |
|          |               | TT+TC vs. CC   | 52.93         | 0.047      | 1.295      |
|          |               | TT vs. CC+CC   | 0.00          | 0.591      | 1.208      |
| NTG      | Random        | T vs. C        | 74.92         | 0.008      | 1.179      |
|          |               | TT vs. CC      | 0.00          | 0.610      | 1.019      |
|          |               | TC vs. CC      | 0.00          | 0.771      | 0.923      |
|          |               | TT+TC vs. CC   | 68.19         | 0.024      | 1.217      |
| By ethnicity |               |                |               |            |                |
| Asian    | Random        | T vs. C        | 60.66         | 0.000      | 1.113      |
|          |               | TT vs. CC      | 31.93         | 0.113      | 1.105      |
|          |               | TC vs. CC      | 35.16         | 0.071      | 1.066      |
|          |               | TT+TC vs. CC   | 58.36         | 0.001      | 1.063      |
|          |               | TT vs. CC+CC   | 52.48         | 0.009      | 1.146      |
| Caucasian| Random        | T vs. C        | 57.90         | 0.004      | 1.139      |
|          |               | TT vs. CC      | 35.06         | 0.102      | 1.088      |
|          |               | TC vs. CC      | 46.01         | 0.035      | 1.094      |
|          |               | TT+TC vs. CC   | 60.39         | 0.003      | 1.195      |
|          |               | TT vs. CC+CC   | 1.848         | 0.428      | 1.087      |

Quantitative Synthesis

MTHFR C677T Polymorphism

Table 3 listed the main results of the meta-analysis of MTHFR C677T polymorphism and glaucoma risk. After the 33 case-control studies were pooled into meta-analysis, no evidence of a significant association between MTHFR C677T polymorphism and glaucoma risk was observed under all genetic models (T vs. C: OR = 1.120, 95% CI 0.994-1.262, P = 0.062, Fig. 2A; TT vs. CC: OR = 1.081, 95% CI 0.899-1.299, P = 0.410; TC vs. CC: OR = 1.033, 95% CI 0.899-1.188, P = 0.646; TT+TC vs. CC: OR = 1.113, 95% CI 0.948-1.306, P = 0.193; TT vs. TC+CC: OR = 1.015, 95% CI 0.876-1.175, P = 0.845).
Fig. 2 Forest plots for the association of MTHFR C677T and A1298C polymorphisms with risk of glaucoma. 

A: MTHFR C677T (allele model: T vs. C); B: MTHFR A1298C (heterozygote model: CA vs. AA)
In the subgroup analysis by glaucoma type, no significant associations with POAG, PACG, PXFG, and NTG subgroups were observed. Moreover, no significant association was found in a subgroup analysis by ethnicity among Asian and Caucasian populations (Table 3). The studies were further stratified based on genotyping technique, source of control subjects and HWE. In the PCR-RFLP group, significantly increased association between MTHFR C677T polymorphism and glaucoma risk were found in the recessive model (TT vs. TC+CC: OR = 1.438, 95% CI 1.056-1.958, P = 0.021). The population based subgroup analysis also revealed that the presence of the MTHFR C677T, which was related to a higher risk of glaucoma under the heterozygote model (TT vs. TC: OR = 1.350, 95% CI 1.012-1.802, P = 0.041). Subgroup analysis of studies in accordance with HWE showed that there was a significant association between MTHFR C677T polymorphism and the increased risk of glaucoma under the allele model (OR = 1.156, 95% CI 1.020-1.309, p = 0.023) (data not shown).

MTHFR A1298C Polymorphism

Table 4 listed the main results of the meta-analysis of MTHFR A1298C polymorphism and glaucoma risk. When all the eligible studies were pooled into the meta-analysis of MTHFR A1298C polymorphism, significantly increased risk of glaucoma was observed in the heterozygote model (CA vs. AA: OR = 0.765, 95% CI 0.626-0.935, p = 0.009, Fig. 2B). Table 4 also summarizes the results of the subgroup analyses by ethnicity and types of glaucoma. When stratified by ethnicity, a significant association between MTHFR A1298C polymorphism and increased risk of glaucoma was detected among Asians (C vs. A: OR = 0.826, 95% CI 0.692-0.987, p = 0.036; CC vs. AA: OR = 0.456, 95% CI 0.268-0.777, p = 0.004; and CA vs. AA: OR = 705, 95% CI 0.541-0.918, p = 0.010) and Caucasians (CC vs. CA+AA: OR = 1.443, 95% CI 1.019-2.044, p = 0.039). In addition, when stratifying by types of glaucoma, we found that MTHFR A1298C was significantly associated with POAG risk under heterozygote model (CA vs. AA: OR = 0.746, 95% CI 0.570-0.976, p = 0.033), but not with PXFG and NTG (Table 4). Moreover, subgroup analysis of studies in agreement with HWE showed that there was a significant association between MTHFR A1298C polymorphism and increased risk of glaucoma under the recessive model (OR = 1.440, 95% CI 1.023-2.026, p = 0.037) (data not shown).

Table 4. Summary risk estimates for association between MTHFR A1298C polymorphism and glaucoma risk

| Subgroup | Genetic Model | Type of Model | Heterogeneity | Odds ratio 95% CI | ZOR | ZOR | Publication Bias |
|----------|---------------|---------------|---------------|------------------|-----|-----|-----------------|
|          |               |               | F (%) | P H | P OR | OR | ZOR | P OR | P Hedges | P Eggers |
| Overall  | C vs. A       | Fixed         | 42.53  | 0.584 | 0.943 | 0.826-1.075 | -0.880 | 0.379 | 0.602 | 0.257 |
|          | CC vs. AA     | Fixed         | 44.50  | 0.702 | 0.878 | 0.627-1.231 | -0.753 | 0.425 | 0.602 | 0.909 |
|          | CA vs. AA     | Fixed         | 0.00   | 0.753 | 0.765 | 0.626-0.935 | -2.610 | 0.009 | 0.602 | 0.666 |
|          | CC+CA vs. AA  | Fixed         | 26.84  | 0.205 | 0.873 | 0.721-1.058 | -1.381 | 0.167 | 0.602 | 0.620 |
|          | CC vs. CA+AA  | Fixed         | 24.92  | 0.222 | 1.083 | 0.824-1.422 | 0.571 | 0.568 | 0.754 | 0.924 |
| By Type  |               |               |       |     |     |               |       |       |       |       |
| POAG     | C vs. A       | Fixed         | 0.00   | 0.507 | 0.939 | 0.787-1.120 | -0.700 | 0.484 | 0.734 | 0.857 |
|          | CC vs. AA     | Fixed         | 38.10  | 0.183 | 1.027 | 0.655-1.611 | 0.115 | 0.908 | 1.000 | 0.767 |
|          | CA vs. AA     | Fixed         | 0.00   | 0.861 | 0.746 | 0.570-0.976 | -2.138 | 0.033 | 1.000 | 0.763 |
|          | CC+CA vs. AA  | Fixed         | 0.00   | 0.837 | 0.832 | 0.643-1.077 | -1.394 | 0.163 | 1.000 | 0.673 |
|          | CC vs. CA+AA  | Fixed         | 26.28  | 0.254 | 1.153 | 0.798-1.666 | 0.757 | 0.449 | 0.308 | 0.539 |
| PXFG     | C vs. A       | Fixed         | 0.00   | 0.449 | 1.179 | 0.844-1.646 | 0.966 | 0.334 | NA    | NA    |
|          | CC vs. AA     | Fixed         | 0.00   | 0.558 | 1.313 | 0.665-2.591 | 0.785 | 0.432 | NA    | NA    |
|          | CA vs. AA     | Fixed         | 0.00   | 0.864 | 0.938 | 0.513-1.715 | -0.217 | 0.836 | NA    | NA    |
|          | CC+CA vs. AA  | Fixed         | 0.00   | 0.670 | 1.050 | 0.601-1.833 | 0.170 | 0.865 | NA    | NA    |
|          | CC vs. CA+AA  | Fixed         | 0.00   | 0.530 | 1.422 | 0.852-2.374 | 1.346 | 0.178 | NA    | NA    |
| NTG      | C vs. A       | Fixed         | 70.83  | 0.664 | 1.204 | 0.608-2.382 | 0.532 | 0.595 | NA    | NA    |
|          | CC vs. AA     | Fixed         | 0.00   | 0.512 | 0.650 | 0.133-3.171 | -0.533 | 0.594 | NA    | NA    |
|          | CA vs. AA     | Fixed         | 0.00   | 0.477 | 0.926 | 0.607-1.413 | -0.356 | 0.722 | NA    | NA    |
|          | CC+CA vs. AA  | Fixed         | 71.59  | 0.061 | 1.178 | 0.783-1.773 | 0.785 | 0.432 | NA    | NA    |
|          | CC vs. CA+AA  | Fixed         | 0.00   | 0.391 | 0.910 | 0.188-4.402 | -0.118 | 0.906 | NA    | NA    |

By Ethnicity

Asians C vs. A Fixed 52.89 0.075 0.826 0.692-0.987 -2.099 0.036 0.220 0.115
Minor Allele Frequencies (MAFs)

The minor allele frequencies (MAFs) of the MTHFR C677T and A1298C polymorphisms by ethnicity are presented in Tables 1 and 2. The allele and genotype distributions of MTHFR C677T and A1298C polymorphisms exhibited ethnic variations. The 677T allele frequencies in the Caucasian and Asians populations were 30.7% (18.4%-43.0%) and 22.75% (9.2%-36.3%), respectively. The 1298C allele frequencies in the Caucasian and Asians populations were 34.3% (11.7%-56.9%) and 17.85% (14.0%-21.7%), respectively. Therefore, the frequencies of the 677T and 1298C alleles in Asians were less than in Caucasians.

Heterogeneity Test and Sensitivity Analyses

There was a significant heterogeneity among these studies for MTHFR C677T polymorphism under allele model comparison (T vs. C: \( P_h = 0.001 \)), homozygote model comparison (TT vs. CC: \( P_h = 0.005 \)) and dominant model comparison (TT + CT vs. CC: \( P_h = 0.001 \)). Then, we assessed the source of heterogeneity by meta-regression analysis. However, we found that ethnicity, glaucoma types, genotyping methods, source of controls and HWE did not contribute to substantial heterogeneity among the meta-analysis (Table 2). Sensitivity analyses were conducted to determine whether modification of the inclusion criteria of the current meta-analysis affected the findings. Although the sample size for cases and controls in all eligible studies ranged from 18 to 243, the pooled ORs were not qualitatively altered by omitting the study of small sample. Three studies (Mabuchi et al., Al-Shahrani et al., and Dixit et al.) were not in HWE; however, the overall association was unchanged after the exclusion of these studies, which indicated that the results from this meta-analysis were statistically robust. Moreover, the heterogeneity test showed that there was no significant between-study heterogeneity in terms of the MTHFR A1298C polymorphism in the overall comparisons and subgroup analyses (Table 3).

Publication Bias

We have used both Begg’s funnel plot and Egger’s test to access the small study effects of articles in literature. The shape of the funnel plots did not reveal an obvious asymmetry. Then, the Egger’s test was used to provide statistical evidence of funnel plot symmetry. Egger’s test found evidence for the publication bias between MTHFR C677T polymorphism and glaucoma risk under the allele model (T vs. C: \( P_{Egger} = 0.052, P_{Egger} = 0.031 \), Fig. 3), homozygote model (TT vs. CC: \( P_{Egger} = 0.010, P_{Egger} = 0.008 \)) and the recessive model (TT vs. CT + CC: \( P_{Egger} = 0.022, P_{Egger} = 0.005 \)). This finding might be a limitation for this meta-analysis because studies with null findings, especially those with small sample size, are less likely to be published. The Duval and Tweedie non-parametric “trim and fill” method was used to adjust for publication bias. Meta-analysis with and without “trim and fill” did not draw a different conclusion, indicating that our results were statistically robust. Moreover, no significant publication bias for MTHFR A1298C polymorphism was found by Egger’s test in the overall or subgroup analyses.
Discussion

To the best of our knowledge, this is the first and most comprehensive meta-analysis assessing the associations of MTHFR C677T and A1298C polymorphisms with risk of different types of glaucoma. A total of 33 case-control studies in 19 publications (3,504 cases and 2,525 controls) and nine case-control studies in six publications (1,073 cases and 775 controls) have investigated the associations of MTHFR C677T and A1298C polymorphisms with glaucoma risk, respectively. Our meta-analysis showed that MTHFR C677T polymorphism was not associated with glaucoma risk. Similar results were observed in the subgroup analyses based on ethnicity and types of glaucoma (POAG, PACC, PEXG, and NTG). However, we have found that the MTHFR A1298C may be associated with an increased glaucoma risk overall and by ethnicity. Moreover, in a subgroup analysis of glaucoma types, MTHFR A1298C polymorphism was significantly associated with an increased risk of POAG, but not with PXFG and NTG subgroups.

Interestingly, stratified analysis according to genotyping technique revealed a significantly increased risk of glaucoma in participants with the C677T polymorphism in those studies involving PCR-RFLP under recessive genetic model (TT vs. TC+CC: OR = 1.438, 95% CI 1.056-1.958, P = 0.021). With the recent advent of sophisticated high-throughput genotyping technologies such as semi nested PCR, the TaqMan allelic discrimination test, or real-time PCR, we may witness a significant progress in the association studies in the future [32]. High sensitivity of real-time PCR makes the technique applicable to very small samples [33]. However, this trend is possible because studies involving Caucasians mainly utilized Real-Time PCR. While, in studies involving Asians, PCR-RFLP was the main genotyping technique. We proposed that the sensitivity and specificity of genotyping techniques are further explored to seek out optimal approaches that could minimize the genotyping errors. Therefore, this result should be carefully interpreted and confirmed by conducting a further analysis of additional published studies. Moreover, the population based subgroup analysis also revealed that the presence of the MTHFR C677T was related to a higher risk of glaucoma under heterozygote genetic model (TC vs. CC: OR = 1.350, 95% CI = 1.012-1.802, P = 0.041). Similarly, Huo et al. suggested that there were significant associations between MTHFR C677T polymorphism and POAG in allelic genetic model and additive genetic model for population-based subgroup, which indicated that the T allele or TT genotype might increase the risk of POAG [34].

Pathogenesis of POAG is a complex process. It is known that genetic factors play an important role in POAG susceptibility [35]. However, most of the molecular mechanisms leading to POAG development are still unknown [36]. It seems that approximately 5% of POAG is currently attributed to a single-gene or Mendelian forms of glaucoma. Gene mutations in various loci have been identified by genetic studies and a genetic basis for glaucoma pathogenesis has been established [18,37]. Although many epidemiological studies have been conducted to assess the roles of MTHFR C677T polymorphism and POAG risk in different populations, results have been inconclusive. Recently, in a case-control study of 144 POAG cases and 280 controls in Saudi Arabia, Al-Sharani et al. indicated that the allele T and genotype CT of MTHFR C677T polymorphism confer risk of POAG, while allele C and CC genotype had a different role [30]. However, four studies did not find an association between MTHFR C677T polymorphism and POAG risk in Iranian, Mexican, Indian and Greek populations [25,27–29]. In 2012, Xu et al. have conducted the first meta-analysis including ten studies with 1,406 cases and 1,216 controls on MTHFR C677T polymorphism [38]. They found no impact of MTHFR C677T polymorphism on POAG susceptibility in the pooled analysis. Since then, a series of better-designed case-control studies on the association between MTHFR C677T polymorphism and POAG were performed. In the current meta-analysis, 16 eligible studies with 2,179 cases and 2,069 controls were identified and analyzed. The present meta-analysis suggested that there was no significant association between MTHFR C677T and POAG risk in the overall comparisons. Consistent with our study, a previous meta-analysis was undergone in 2015, which included 13 studies with 1,970 POAG patients and 1,712 control subjects, suggesting that the MTHFR C677T was
not associated with increased genetic susceptibility to POAG [39]. However, we found out they wrongly included one study evaluated about the \textit{MTHFR C677T} polymorphism and PACG risk in their meta-analysis. Our literature search was more thorough, containing four more articles, which increased the total number of cases and controls, thus, providing a greater power to our conclusions. Moreover, we used one more genetic model, the allele genetic model, to gain a more comprehensive and accurate understanding of the \textit{MTHFR C677T} polymorphism association.

Assessing heterogeneity in the meta-analysis of genetic associations is critical for model selection and interpretation of the results. On the other hand, heterogeneity and publication bias might influence the results of the meta-analysis. It is well known that different factors, such as population stratification, source of controls, population size, deviation from Hardy–Weinberg equilibrium, and other covariates could be the source of heterogeneity. In the current meta-analysis, moderate between-study heterogeneity was detected across studies under allele, heterozygote and dominant genetic models for \textit{MTHFR C677T} polymorphism and thus we selected the random-effects model to summarize the ORs. Therefore, we performed a meta-regression analysis to find the source of between-study heterogeneity. The results showed that ethnicity, glaucoma types, genotyping methods, source of controls and HWE status did not contribute to substantial between-study heterogeneity in the current meta-analysis.

It was obvious that some limitations of this meta-analysis should be considered. First, the sample size reported in literature is still relatively small and might not provide sufficient power to estimate the association between the null \textit{MTHFR A1298C} polymorphism and the glaucoma risk. Second, the language of the publications was limited to English. Third, the current meta-analysis was based predominantly on Asian and Caucasian research. No study from other parts of the world was found, such as the Africans. This suggested a partial result that is only relevant to the Asian and Caucasian subgroups. Forth, the existence of between-study heterogeneity in some comparisons might compromise the reliability of conclusion. Finally, glaucoma is a multifactorial disease that results from complex interactions between various genetic and environmental factors. Due to the unavailability of other detailed information, our results were based on single-factor estimates without adjustments for other risk factors. Further evaluation of glaucoma risk should pay more attention to the potential interactions among gene–gene, gene–environment, and even different polymorphism of the \textit{MTHFR} gene and other loci. Despite these limitations, our meta-analysis had some clear advantages. Our meta-analysis contained the largest sample size to date to assess the association between the \textit{MTHFR C677T} and \textit{A1298C} polymorphisms and glaucoma risk.

In summary, the current meta-analysis indicated that \textit{MTHFR C677T} might not be associated with the glaucoma risk, and yet the \textit{MTHFR A1298C} polymorphism may be a risk factor for glaucoma. In the future, large sample studies should be warranted to investigate the association of \textit{MTHFR C677T} and \textit{A1298C} polymorphisms with glaucoma, and to examine the potential gene-gene and gene-environment interactions.

Funding

No specific funding was obtained to support the conduct of this study.

Conflict of interest

The authors declared that there is no conflict of interest.

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