Methylenetetrahydrofolate reductase genetic polymorphism and the risk of diabetic nephropathy in type 2 diabetic patients

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Abstract
Background: As indicated by numerous studies, there exists a relationship between the polymorphism of methylenetetrahydrofolate reductase (MTHFR) and susceptibility to diabetic nephropathy (DN) in various populations; nonetheless, the findings remain inconsistent. Therefore, we carried out a meta-analysis to determine the relationship between the MTHFR gene polymorphism and DN susceptibility.

Materials and method: Related studies were identified from PubMed, Cochrane Library, EMBASE, and the China National Knowledge Infrastructure database (time period: from building the library to October 2019). The strength of the association was examined using odds ratios (ORs) with 95% confidence intervals (95% CIs).

Results: The findings illustrated that the C677T gene polymorphism was significantly associated with an enhanced susceptibility to DN compared to that with diabetes mellitus in allelic (OR = 1.64, 95% CI = 1.34–2.00, P < .001), dominant (OR = 1.85, 95% CI = 1.40–2.46, P < .001), codominant (heterozygote: OR = 1.67, 95% CI = 1.27–2.12, P < .001; homozygote: OR = 2.55, 95% CI = 1.82–3.57, P < .001), and recessive (OR = 1.89, 95% CI = 1.50–2.38, P < .001) models of the overall population. Moreover, as compared with the healthy controls, a significantly augmented susceptibility to DN was found in all 5 genetic comparison models (allelic: OR = 2.06, 95% CI = 1.58–2.67, P < .001; dominant: OR = 2.52, 95% CI = 1.73–3.69, P < .001; codominant: OR = 3.78, 95% CI = 2.50–5.70, P < .001; recessive: OR = 2.41, 95% CI = 1.96–2.97, P < .001). Furthermore, stratifying data by ethnicity revealed substantially augmented vulnerability to DN in not only Caucasian but also Asian populations.

Conclusion: The present study suggests that the C677T polymorphism was associated with an augmented susceptibility to DN.

Abbreviations: CI = confidence interval, DM = diabetes mellitus, DN = diabetic nephropathy, HWE = Hardy-Weinberg equilibrium, MTHFR = methylenetetrahydrofolate reductase, ORs = odds ratios, T2D = type 2 diabetes.

Keywords: C677T, diabetic nephropathy, meta-analysis, methylenetetrahydrofolate reductase, polymorphism

1. Introduction

Type 2 diabetes (T2D) is a highly prevalent chronic disease that affects millions of people globally, accordingly giving rise to substantial health outcomes coupled with elevated healthcare expenditures.[1,2] It is widely accepted that vascular complications constitute the primary causes resulting in diabetes mortality as well as disability. Diabetic nephropathy (DN) is considered among the most common microangiopathic complications of T2D as well as a key cause leading to end-stage renal failure, which affects more than 20% of patients with T2D.[3,4] Robust evidence in studies on the candidate gene relationship and connections suggest the vulnerability of patients to DN.[5] Reportedly, the genetic variants in genes encoding methylenetetrahydrofolate reductase (MTHFR) are likely to confer vulnerability to DN.[6] MTHFR is a major regulatory enzyme in the metabolism of folate as well as homocysteine.[7] This enzyme catalyzes the remethylation of homocysteine to methionine; additionally, the lack of MTHFR is likely associated with an increase in plasma homocysteine that, consequently, is associated with an augmented susceptibility to vascular diseases, including DN.[8,9] Moreover, the C677T variant frequently found in the gene encoding the folate-metabolizing enzyme MTHFR is considered as the most renowned genetic determinant that affects suboptimal health outcomes and consequently, is associated with an augmented vulnerability to DN.[10]
In 1998, Neugebauer, together with colleagues, proposed for the first time a correlation between the polymorphism of MTHFR C677T with the susceptibility to DN.[11] Consequently, numerous studies have attempted to analyze the effect of the MTHFR C677T polymorphism on DN susceptibility in different populations; nonetheless, no apparent agreement was attained among the results. Consequently, we implemented an updated meta-analysis with current findings to clarify the effects of the MTHFR C677T polymorphism on the susceptibility to DN by using eligible data obtained from the published case-control studies.

2. Materials and methods

2.1. Search strategy

We conducted a computerized literature search in not only PubMed but also in EMBASE, Cochrane Library, and the China National Knowledge Infrastructure database (time period: up to October 2019). MeSH and the title/abstract were used for finding the qualifying case-control studies in accordance with the following keywords: “methylene tetrahydrofolate reductase OR MTHFR OR C677T” AND “polymorphism* OR mutation* OR variant* OR genotype*” AND “diabetic nephropathy OR diabetes nephropathy.” Our study was approved by the Ethics Committee of West Anhui Health Vocational College.

2.2. Inclusion and exclusion criteria

Qualifying studies aligned with the following criteria: the studies
1. estimated the association existing between the polymorphism of MTHFR C677T and the susceptibility to DN;
2. provided sufficient information on C677T genotype frequencies for the determination of odds ratios (ORs) with 95% confidence intervals (95% CIs) among human individuals with DN; and
3. used a case-control, nested case-control, cross-sectional research design.

Exclusion criteria were as follows:
1. research works without comprehensive genotype data;
2. case studies, reviews, and letters; and
3. duplicate studies.

2.3. Data extraction

Two authors independently extracted the relevant information in accordance with the abovementioned inclusion and exclusion criteria. In addition, the data presented herein were extracted from all included studies: primary author, publication year, country, ethnicity, detection method of genotypes, and the frequency of genotypes among DN patients and controls. Disagreements were resolved by means of discussion between the 2 authors until an agreement was attained.
2.4. Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was assessed between the controls using the $\chi^2$ test or Fisher exact test; in addition, a $P$-value above 0.001 demonstrated the fact that the population was in genetic equilibrium. The ORs and corresponding 95% CIs were utilized to quantify the strength of the relationship existing between the MTHFR C677T polymorphism and the susceptibility to DN. Furthermore, the importance of the accumulated OR

### Table 1

**Characteristics of included study.**

| Author       | Year | Country | Ethnicity | Detection method | CC  | CT  | TT  | CC  | CT  | TT  | HWE  |
|--------------|------|---------|-----------|------------------|-----|-----|-----|-----|-----|-----|------|
| Wang        | 2001 | China   | Asian     | PCR              | 20  | 34  | 28  | 32  | 34  | 13  | 0.502|
| Sun         | 2001 | China   | Asian     | PCR-RFLP         | 8   | 20  | 11  | 24  | 13  | 9   | 0.008|
| Chen        | 2004 | China   | Asian     | PCR              | 6   | 21  | 14  | 18  | 24  | 8   | 0.039|
| Yang        | 2001 | China   | Asian     | PCR-RFLP         | 17  | 27  | 23  | 26  | 28  | 8   | 0.015|
| Xu          | 2003 | China   | Asian     | PCR-RFLP         | 15  | 33  | 21  | 24  | 21  | 9   | 0.853|
| Wen         | 2008 | China   | Asian     | PCR-RFLP         | 22  | 50  | 23  | 27  | 25  | 5   | 0.817|
| Lin         | 2009 | China   | Asian     | PCR-RFLP         | 56  | 36  | 47  | 93  | 22  | 5   | <0.001|
| Yue         | 2006 | China   | Asian     | PCR-RFLP         | 23  | 55  | 34  | 43  | 76  | 21  | 0.903|
| Sun         | 2013 | China   | Asian     | PCR-RFLP         | 35  | 53  | 14  | 43  | 53  | 8   | 0.128|
| Yoshikawa   | 2004 | Japan   | Asian     | PCR-RFLP         | 21  | 13  | 6   | 71  | 107 | 29  | 0.261|
| Ukic        | 2009 | Turkey  | Caucasian | PCR              | 6   | 16  | 0   | 22  | 8   | 0   | 0.390|
| Sun         | 2004 | China   | Asian     | PCR              | 45  | 53  | 26  | 57  | 23  | 16  | <0.001|
| Ramanathan  | 2019 | India   | Asian     | PCR              | 72  | 71  | 2   | 81  | 19  | 0   | 0.294|
| Nemr(a)     | 2010 | Lebanon | Caucasian | PCR-RFLP        | 78  | 104 |
| Nemr(b)     | 2010 | Bahrain | Caucasian | PCR-RFLP        | 158 | 58  |
| Dan         | 2012 | China   | Asian     | PCR-RFLP         | 22  | 26  | 12  | 29  | 28  | 3   | 0.177|
| Movva       | 2011 | India   | Asian     | PCR-RFLP         | 53  | 30  | 0   | 34  | 32  | 0   | 0.509|
| Odawara     | 1999 | Japanese| Asian     | PCR              | 52  | 65  | 26  | 38  | 68  | 25  | 0.579|
| Bogle       | 2007 | Germany | Caucasian | PCR              | 188 | 219 | 32  | 64  | 69  | 15  | 0.566|
| Engli       | 2007 | Turkey  | Caucasian | PCR              | 26  | 20  | 1   | 25  | 25  | 6   | 0.172|
| El-Baz      | 2012 | Egypt   | Caucasian | PCR-RFLP        | 32  | 46  | 24  | 78  | 19  | 3   | 0.189|
| Bluthner    | 1999 | Poland  | Caucasian | PCR              | 74  | 50  | 23  | 63  | 65  | 18  | 0.709|
| Kiszek      | 2004 | Poland  | Caucasian | PCR              | 77  | 65  | 29  | 82  | 58  | 15  | 0.237|
| Ma          | 2019 | China   | Asian     | TaqMan           | 48  | 166 | 107 | 79  | 169 | 86  | 0.82|
| Shpichnetsky| 2000 | Israel  | Caucasian | PCR              | 23  | 22  | 10  | 21  | 16  | 6   | 0.517|
| Sun         | 2001 | China   | Asian     | PCR-RFLP         | 29  | 55  | 28  | 41  | 35  | 18  | 0.008|
| Shcherbak   | 1999 | Russia  | Caucasian | PCR              | 19  | 21  | 11  | 56  | 29  | 5   | 0.113|
| Mitraoui    | 2007 | Tunisia | Caucasian | PCR-RFLP        | 11  | 56  | 26  | 152 | 79  | 36  | <0.001|
| Rahimi      | 2010 | Iran    | Caucasian | PCR              | 60  | 63  | 17  | 45  | 26  | 2   | 0.438|

CC = wild genotype, CT = heterozygous genotype, DM = diabetes mellitus, DN = diabetic nephropathy, HWE = Hardy-Weinberg equilibrium, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, TT = homozygous genotype.

### Table 2

**The results of meta-analysis for different populations in various genotype models (DN vs DM).**

| Genetic model | Population | Number of studies | $I^2$ | $P$  | $F$  | Model | Pooled OR | 95% CI  | $P$-meta | Test of Egger |
|---------------|------------|------------------|------|------|------|-------|-----------|---------|----------|-------------|
| **T vs. C**   | Overall    | 24               | 0.83 | <.001| R    | 1.64  | 1.34–2.00 | <.001   | 0.576    |
|               | Asian      | 13               | 0.80 | <.001| R    | 1.54  | 1.23–1.92 | <.001   | 0.317    |
|               | Caucasian  | 11               | 0.80 | <.001| R    | 1.76  | 1.24–2.50 | <.001   |          |
| **TC vs. CC** | Overall    | 24               | 0.70 | <.001| R    | 1.67  | 1.27–2.21 | <.001   | 0.148    |
|               | Asian      | 13               | 0.72 | <.001| R    | 1.52  | 1.07–2.18 | .02     |          |
|               | Caucasian  | 11               | 0.86 | <.001| R    | 1.85  | 1.20–2.86 | .005    |          |
| **TT vs. CC** | Overall    | 22               | 0.72 | <.001| R    | 2.55  | 1.82–3.57 | <.001   | 0.628    |
|               | Asian      | 12               | 0.56 | <.001| R    | 2.33  | 1.66–3.27 | <.001   |          |
|               | Caucasian  | 10               | 0.82 | <.001| R    | 2.76  | 1.45–5.24 | .002    |          |
| **TT+TC vs.** | Overall    | 24               | 0.85 | <.001| R    | 1.85  | 1.40–2.46 | <.001   | 0.235    |
| **CC**        | Overall    | 22               | 0.86 | <.001| R    | 1.69  | 1.19–2.41 | <.003   |          |
|               | Asian      | 12               | 0.21 | <.001| R    | 1.73  | 1.38–2.17 | <.001   |          |
|               | Caucasian  | 10               | 0.63 | <.001| R    | 2.07  | 1.32–3.24 | <.001   |          |

CC = wild genotype, CI = confidence interval, CT = heterozygous genotype, DM = diabetes mellitus without nephropathy, DN = diabetic nephropathy, F = fixed-effect model, OR = odds ratio, $P$-meta = $P$-value of pooled effect, R = random-effect model, TT = homozygous genotype.
was examined using a Z-test, where \( P < .05 \) suggested the statistical significance. The between-study heterogeneity was assessed with the \( Q \) statistic, Labbe plot, and \( I^2 \) statistic.\(^{12,13}\) The fixed-effect framework (Mantel-Haenszel method) was conducted at \( \Phi > 0.1 \) or at \( F < 50\% \);\(^{14}\) otherwise, the random-effect framework (DerSimonian-Laird method) was applied.\(^{15}\) Moreover, subgroup analysis was conducted on the basis of ethnicity. The sensitivity analysis was performed through omitting each study individually to evaluate the robustness of the findings. Begg funnel plot as well as Egger test were undertaken to evaluate latent publication bias.\(^{16,17}\) All statistical analyses were carried out using STATA software version 15.0 for Windows.

3. Results

3.1. Characteristics of the studies

Figure 1 sheds light on the literature search process. An aggregate of 165 pertinent research works was formed from the preliminary search of databases. Four replicated publications were removed in the initial screening. After screening titles and abstracts, 137 unrelated articles were excluded. Moreover, the remaining papers were subjected to a full-text review by 2 independent authors. Eventually, 28 qualifying studies were included in the current study.\(^{16,18–44}\) Among 8787 participants, 4154 were Asian while 4633 participants were Caucasian. Among all 28 research populations, the allocations of the MTHFR C677T polymorphism in the controls were in alignment with HWE, except for three research studies.\(^{17,31,40}\) Table 1 summarizes the attributes of the registered research works as well as the HWE examination findings.

3.2. The MTHFR C677T polymorphism and DN (DN vs diabetes mellitus [DM])

The heterogeneity was assessed with the \( Q \) statistic, Labbe plot, and \( I^2 \) statistic in 5 genetic frameworks. As presented in Table 2, significant heterogeneity was detected in 5 genetic models; accordingly, the random-effect framework was adopted in this analysis. The Labbe plots for the MTHFR C677T polymorphism in the allelic and recessive models are presented in Figure 6. As the findings suggested, there was a significant relationship between the polymorphism of C677T and an augmented susceptibility to DN compared with that to DM in allelic (\( OR = 1.64, 95\% CI = 1.34–2.00, P < .001 \)), dominant (\( OR = 1.85, 95\% CI = 1.40–2.46, P < .001 \)), codominant (heterozygote: \( OR = 1.67, 95\% CI = 1.27–2.21, P < .001 \); homozygote: \( OR = 2.55, 95\% CI = 1.82–3.57, P < .001 \)), and recessive (\( OR = 1.89, 95\% CI = 1.50–2.38, P < .001 \)) frameworks in the populations in general (Fig. 2).

Figure 2. Forest plots for the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility (compared diabetic nephropathy group with diabetes mellitus group). (A) allelic model; (B) homozygote model; (C) heterozygote model; (D) recessive model.
earlier comparisons, we carried out subgroup analysis based on ethnicity, and no substantial changes were observed in the risk estimations in all genetic comparison frameworks. The stratified analysis based on ethnicity revealed significantly enhanced susceptibility to DN in Caucasian and Asian populations, as shown in Figure 3. In addition, three of the research works\[27,31,40\] had genotype distributions of the C677T polymorphism in DM controls that deviated from HWE; however, the accumulated ORs still reached significance in all genetic comparison models after excluding these three research studies. The key results are shown in Table 2.

### 3.3. The MTHFR C677T polymorphism and DN (DN vs healthy control)

The influence of the MTHFR C677T polymorphism on DN susceptibility was evaluated in 18 research works. Significant heterogeneity was observed in the genetic comparison models except for the recessive genetic model; accordingly, the random-effect model was adopted to evaluate the correlation existing between the MTHFR C677T polymorphism and DN susceptibility. Furthermore, the Labbe plots for the MTHFR C677T polymorphism in the allelic and recessive models are shown in Figure 6. The overall analysis shed light on the fact that the MTHFR C677T polymorphism had a significant correlation with an augmented susceptibility to DN in all five genetic comparison frameworks (allelic model: \( OR = 2.06,\) 95% \( CI = 1.58–2.67\), \( P < .001\); dominant model: \( OR = 2.52,\) 95% \( CI = 1.73–3.69\), \( P < .001\); codominant model: \( OR = 3.78,\) 95% \( CI = 2.50–5.70\), \( P < .001\); recessive model: \( OR = 2.41,\) 95% \( CI = 1.96–2.97\), \( P < .001\)), as shown in Figure 4. Owing to the substantial between-study heterogeneity determined in the earlier comparisons, subgroup analysis was carried out on the basis of ethnicity, and no substantial change was observed in the risk estimations in all genetic comparison frameworks. The subgroup analysis based on ethnicity showed a substantial increase in susceptibility to DN among Asian populations in the 5 genetic comparison frameworks; however, a significant association in Caucasian populations was found only in the recessive genetic model (\( OR = 2.34,\) 95% \( CI = 1.68–3.24,\) \( P < .001\)), as presented in Figure 5. Moreover, in 2 studies\[31,40\] the genotype distributions of the C677T polymorphism in DM controls deviated from HWE, and the accumulated ORs still reached significance in all genetic comparison models after excluding these 3 studies.

### 3.4. Sensitivity analysis and publication bias

To assess whether a sole research work could impact the final ORs, each separate research work was eliminated once, after which the data was repooled. The analysis findings illustrated that the accumulated ORs were not affected by the deletion of individual studies, as shown in Figure 7. Begg funnel plot, together with Egger test, was employed to evaluate the publication partiality. All plots were observed as having...
Figure 4. Forest plots for the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility (compared diabetic nephropathy group with healthy control group). (A) allelic model; (B) homozygote model; (C) heterozygote model; (D) recessive model.

Figure 5. Forest plots for the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility stratified analyses according to ethnicity (compared diabetic nephropathy group with healthy control group). (A) allelic model; (B) homozygote model; (C) heterozygote model; (D) recessive model.
approximate symmetry, which suggested that no evident publication bias was present. Moreover, the main results are presented in Figure 8 and Tables 2 and 3.

4. Discussion

Diabetes and its related complications represent a substantial health and economic load; in addition, given the rising epidemics of obesity as well as diabetes among children and young people, the occurrence of diabetes is anticipated to continue growing. Furthermore, the pathophysiology of DN remains ambiguous, thus requiring further investigation. There appears to be an inherited predisposition for DN; in addition, there are some candidate genes that have been reproducibly connected to DN. Gene studies are likely to offer worthwhile information about the pathobiology of DN as well as the latest targets for its therapy.\textsuperscript{[1]}

The pathogenesis of DN is multifactorial, and the high level of plasma homocysteine is considered a key risk factor for the development of DN.\textsuperscript{[45]} Moreover, homocysteine is considered as an intermediary sulfur compound that contains the product of methionine metabolism, whereas its levels are influenced by the levels of vitamin B12 and folic acid.\textsuperscript{[46]} MTHFR constitutes a major regulatory enzyme in homocysteine and folate metabolism.\textsuperscript{[7]} The polymorphism of MTHFR C677T is likely to exert an effect on the step in homocysteine metabolism in which it is involved.\textsuperscript{[7,46]} The homozygous variants of MTHFR C677T have higher levels of homocysteine, whereas the heterozygous variants have moderately augmented levels of homocysteine in comparison with the homozygous wild-type genotype.\textsuperscript{[10,47]} Accordingly, there exists biological evidence for the correlation of the polymorphism of MTHFR C677T with DN susceptibility.

In 1998, Neugebauer, together with colleagues, first proposed a correlation between the polymorphism of MTHFR C677T and

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**Figure 6.** Labbe plots of the included studies focusing on the association between MTHFR C677T polymorphism and diabetic nephropathy susceptibility. Compared DN group with diabetes mellitus group in allelic model (A), and recessive model (B); compared DN group with healthy control group in allelic model (C), and recessive model (D). DN = diabetic nephropathy.
Figure 7. Sensitivity analysis for the included studies focusing on the relationship of the polymorphism of MTHFR C677T on the susceptibility to diabetic nephropathy. Compared DN group with diabetes mellitus group in allelic model (A), and recessive model (B); compared DN group with healthy control group in allelic model (C), and recessive model (D). DN = diabetic nephropathy.

Figure 8. Begg funnel plots of publication bias for the relationship of the polymorphism of MTHFR C677T on the susceptibility to diabetic nephropathy. Compared DN group with diabetes mellitus group in allelic model (A), and recessive model (B); compared DN group with healthy control group in allelic model (C), and recessive model (D). DN = diabetic nephropathy.
the susceptibility to DN, and the findings illustrated that this polymorphism likely contributes to the development of DN.[11] Consequently, Chang et al carried out a meta-analysis for this association, which indicated that the polymorphism of MTHFR C677T might influence the susceptibility to DN in the Chinese population.[48] In 2016, Xiong and colleagues examined only Chinese studies examining the correlation of the polymorphism of MTHFR C677T with the susceptibility to DN.[49] To attain a more accurate approximation of this correlation, a meta-analysis was carried out in the present study. To our knowledge, our study constitutes the most detailed research addressing the association between the MTHFR C677T polymorphism and DN susceptibility. Moreover, 28 studies involving 8787 participants were included in this analysis. Overall, we elucidated that the polymorphism of MTHFR C677T substantially augmented the susceptibility to DN in not only Asian, but also Caucasian populations.

This study has some limitations. Firstly, the sample size of some studies was limited, which might give rise to bias in the results when assessing the correlation of the polymorphism of MTHFR C677T with the susceptibility to DN. Secondly, the present study was statistically heterogenic, although this is highly frequent in meta-analyses of genetic correlations. Hence, we implemented subgroup analysis to identify all determinants that contributed to heterogeneity. Thirdly, other determinants that are likely to affect the correlation of the MTHFR C677T polymorphism with the susceptibility to DN, such as sex, environment, and lifestyle, could not be analyzed due to a lack of genuine data. Ultimately, only published studies were included in this analysis. Moreover, unpublished works and further studies may be capable of altering our findings. Based on the abovementioned reasons, the pooled estimates of our meta-analysis require careful interpretation.

5. Conclusions

To summarize, the present study suggests that the MTHFR C677T polymorphism is likely to be related to an augmented susceptibility to DN in not only Asian but also Caucasian populations. Nonetheless, prospective studies with effective designs and extensive sample sizes might be beneficial for the validation of this association in various ethnicities.

Author contributions

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References

[1] Guasch-Ferré M, Hu R, Toledo E, et al. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care 2016;39:833–46.
[2] Arneth B, Arneth R, Shams M. Metabolomics of type 1 and type 2 diabetes. Int J Mol Sci 2019;20:2467.
[3] Xue R, Gui D, Zheng L, et al. Mechanistic insight and management of diabetic nephropathy: recent progress and future perspective. J Diabetes Res 2017;2017:1839809.
[4] Thomas MC, Cooper ME, Zimmer P. Changing epidemiology of type 2 diabetes mellitus and associated chronic kidney disease. Nat Rev Nephrol 2016;12:73–81.
[5] Brennan E, McEvoy C, Sadlier D, et al. The genetics of diabetic nephropathy. Genes (Basel) 2013;4:596–619.
[6] Ma L, Jiang Y, Kong X, et al. Interaction of MTHFR C677T polymorphism with smoking in susceptibility to diabetic nephropathy in Chinese men with type 2 diabetes. J Hum Genet 2019;64:23–8.
[7] Liew SC, Gupta ED. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. Eur J Med Genet 2015;58:1–8.
Hiraoka M, Kagawa Y. Genetic polymorphisms and folate status.

Mantel N, Haenszel W. Statistical aspects of the analysis of

Ksiazek P, Bednarek-Skublewska A, Buraczynska M. The C677T methylenetetrahydrofolate reductase gene polymorphism as a risk factor for diabetic nephropathy in NIDDM patients. Lancet 1998;352:629–34.

Zintzaras E, Ioannidis JP. HEGESMA: genome search meta-analysis and heterogeneity testing. Bioinformatics 2005;21:3672–3.

Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–58.

Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719–48.

Dersimonian R, Laird N. Meta-analysis in clinical trials revisited. Contemp Clin Trials 2013;45:139–45.

Begg CB, Mazandarani M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50:1088–101.

Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.

Shcherbak NS, Shudina AM, Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.

Książek P, Bednarek-Skublewska A, Buraczynska M. The C677T methylenetetrahydrofolate reductase gene mutation and nephropathy in type 2 diabetes mellitus. Med Sci Monit 2004;10:BR47–51.

Blüthner M, Brüngens A, Schmide S, et al. Association of methylenetetrahydrofolate reductase gene polymorphism and diabetic nephropathy in type 2 diabetes? Nephrol Dial Transplant 1999;14:36–7.

Odawara M, Yamashita K. A common mutation of the methylenetetrahydrofolate reductase gene as a risk factor for diabetic nephropathy. Diabetologia 1999;42:631–2.

Sun J, Xu Y, Zhu Y, et al. An association study of methylenetetrahydrofolate reductase gene polymorphism with diabetic nephropathy. J Nephrol Dial Transplant 2001;10:33–6.

Shpichetsky V, Raz I, Friedlander Y, et al. The association between two common mutations C677T and A1298C polymorphisms of the MTHFR gene and diabetic nephropathy in Turkish type 2 diabetic patients with and without nephropathy. Diabetes Metab Res Rev 2007;23:621–4.

Moova S, Alluri RV, Venkatasubramanian S, et al. Association of methylenetetrahydrofolate reductase C677T genotype with type 2 diabetes mellitus patients with and without renal complications. Genet Test Mol Biomarkers 2011;15:257–61.

Mitraou N, Ezzidi I, Chaib M, et al. MTHFR C677T and A1298C gene polymorphisms and hyperhomocysteinemia as risk factors of diabetic nephropathy in type 2 diabetes patients. Diabetes Res Clin Pract 2007;75:99–106.

Nemr R, Salman RA, Jawad LH, et al. Differential contribution of MTHFR C677T genotype on survival in type 2 diabetes patients with end-stage diabetic nephropathy. Nephrol Dial Transplant 2007;22:154–62.

El-Baz R, Settin A, Ismaeel A, et al. MTHFR C677T, A1298C and ACE I/D polymorphisms as risk factors for diabetic nephropathy among type 2 diabetic patients. J Renin Angiotensin Aldosterone Syst 2012;13:472–7.

Eroglu Z, Erdogan M, Tertik A, et al. The relationship of the methylenetetrahydrofolate reductase C677T gene polymorphism in Turkish type 2 diabetic patients with and without nephropathy. Diabetes Metab Res Rev 2007;23:621–4.

Rahimi Z, Hasanvand A, Felehgari V. Interaction of MTHFR 1298C with ACE D allele augments the risk of diabetic nephropathy in Western Iran. DNA Cell Biol 2012;31:553–9.

Sun J, Xu Y, Zhu Y, et al. Genetic polymorphism of methylenetetrahydrofolate reductase as a risk factor for diabetic nephropathy in Chinese type 2 diabetic patients. Diabetes Res Clin Pract 2004;64:185–90.

Wang LQ, Wang JY, Xue YM, et al. Relationship between methylenetetrahydrofolate reductase gene polymorphism and diabetic nephropathy. Chin J Genet Med 2001;18:226–8.

Yue H, Liu J, Kang WJ, et al. Relationship between plasma level of homocysteine and urine microalbumin in incipient type 2 diabetic nephropathy. Chin J Gas R 2006;35:725–9.

Sun L, Wang SQ, Wang XX, et al. Association study between MTHFR C677T polymorphism and levels of Scr and BUN in diabetic nephropathy patients. Chin J Health Lab Technol 2011;23:1167–9.

Boger CA, Stubarus M, Haak T, et al. Effect of MTHFR C677T genotype on survival in type 2 diabetes patients with end-stage diabetic nephropathy. Nephrol Dial Transplant 2002;17:e6839.

Chang W-w, Zhang L, Yao Y-s, et al. Methylenetetrahydrofolate reductase C677T variant to the risk of diabetic nephropathy in Lebanese and Bahraini Arabs. Clin Chem Lab Med 2010;48:1091–4.

Bahrami Z, Hasanvand A, Felehgari V. Interaction of MTHFR 1298C with ACE D allele augments the risk of diabetic nephropathy in Western Iran. DNA Cell Biol 2012;31:553–9.

Sun J, Xu Y, Zhu Y, et al. Genetic polymorphism of methylenetetrahydrofolate reductase as a risk factor for diabetic nephropathy in Chinese type 2 diabetic patients. Diabetes Res Clin Pract 2007;75:99–106.

Nemr R, Salman RA, Jawad LH, et al. Differential contribution of MTHFR C677T genotype on survival in type 2 diabetes patients with end-stage diabetic nephropathy. Nephrol Dial Transplant 2007;22:154–62.

El-Baz R, Settin A, Ismaeel A, et al. MTHFR C677T, A1298C and ACE I/D polymorphisms as risk factors for diabetic nephropathy among type 2 diabetic patients. J Renin Angiotensin Aldosterone Syst 2012;13:472–7.

Eroglu Z, Erdogan M, Tertik A, et al. The relationship of the methylenetetrahydrofolate reductase C677T gene polymorphism in Turkish type 2 diabetic patients with and without nephropathy. Diabetes Metab Res Rev 2007;23:621–4.

Rahimi Z, Hasanvand A, Felehgari V. Interaction of MTHFR 1298C with ACE D allele augments the risk of diabetic nephropathy in Western Iran. DNA Cell Biol 2012;31:553–9.

Sun J, Xu Y, Zhu Y, et al. Genetic polymorphism of methylenetetrahydrofolate reductase as a risk factor for diabetic nephropathy in Chinese type 2 diabetic patients. Diabetes Res Clin Pract 2004;64:185–90.

Ukinc K, Eroglu Z, Erdogan M, Tertik A, et al. MTHFR C677T and A1298C gene polymorphisms and hyperhomocysteinemia as risk factors of diabetic nephropathy in type 2 diabetes patients. Diabetes Res Clin Pract 2007;75:99–106.

Nemr R, Salman RA, Jawad LH, et al. Differential contribution of MTHFR C677T genotype on survival in type 2 diabetes patients with end-stage diabetic nephropathy. Nephrol Dial Transplant 2007;22:154–62.

Chang W-w, Zhang L, Yao Y-s, et al. Methylenetetrahydrofolate reductase C677T gene mutation and hyperhomocysteinemia as a novel risk factor for diabetic nephropathy. Endocrine 2009;36:255–61.

Meza Letelier CE, San Martin Ojeda CA, Ruiz Provoste JJ, et al. Pathophysiology of diabetic nephropathy: a literature review. Medwave 2017;17:e6389.

Fowler B. Disorders of homocysteine metabolism. J Inherit Metab Dis 1999;22:270–85.

Rozen R. Genetic predisposition to hyperhomocysteinemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). Thromb Haemost 1999;78:523–6.

Chang W-w, Zhang L, Yao Y-s, et al. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and susceptibility to diabetic nephropathy in Chinese type 2 diabetic patients: a meta-analysis. Renal Failure 2013;35:1038–43.

Xiong X, Lin XK, Xiao X, et al. Association between MTHFR C677T polymorphism and diabetic nephropathy in the Chinese population: an updated meta-analysis and review. Nephrol (Carlton) 2016;21:5–12.